

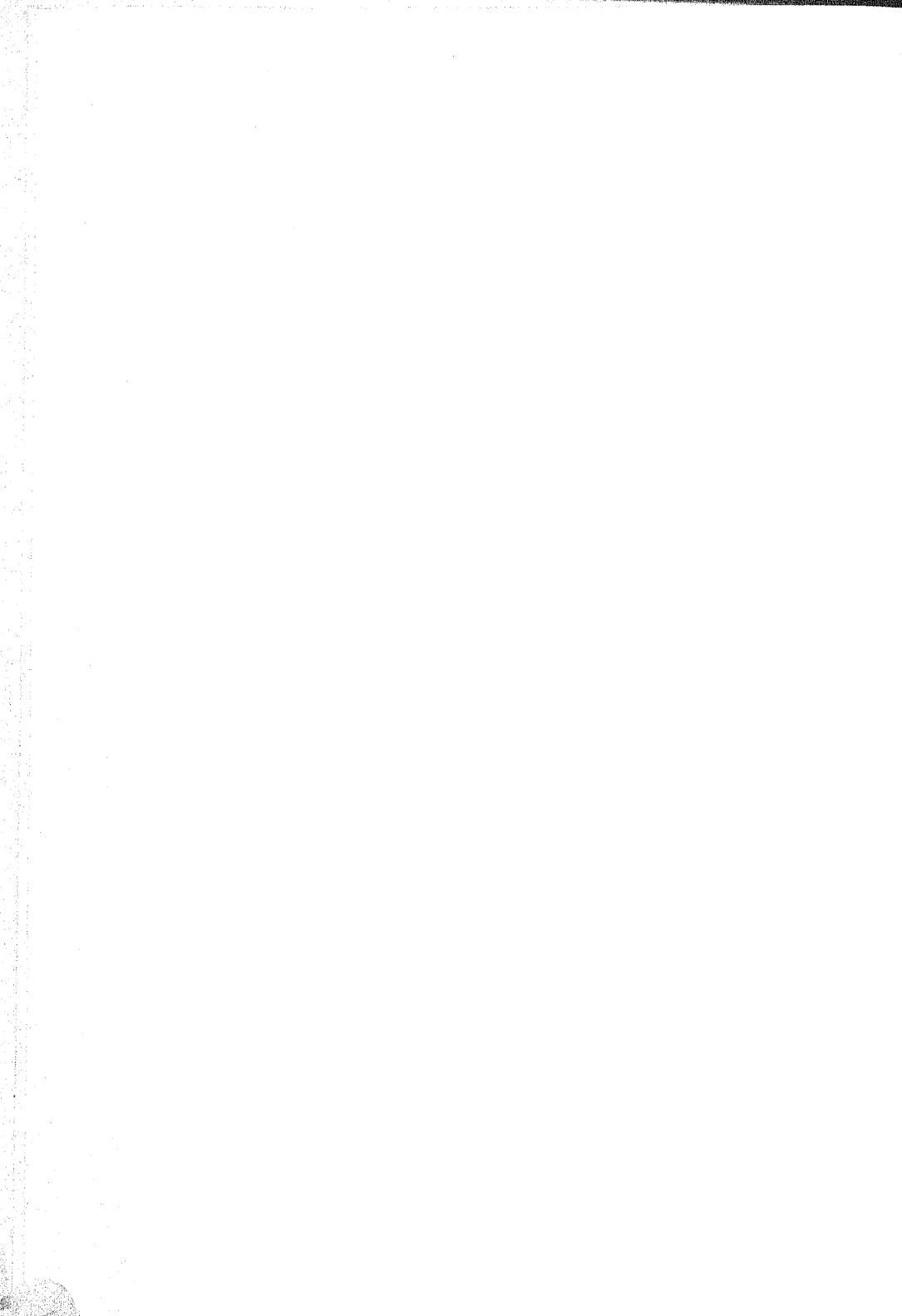
THIN-LAYER

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With a Foreword by
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Foreword

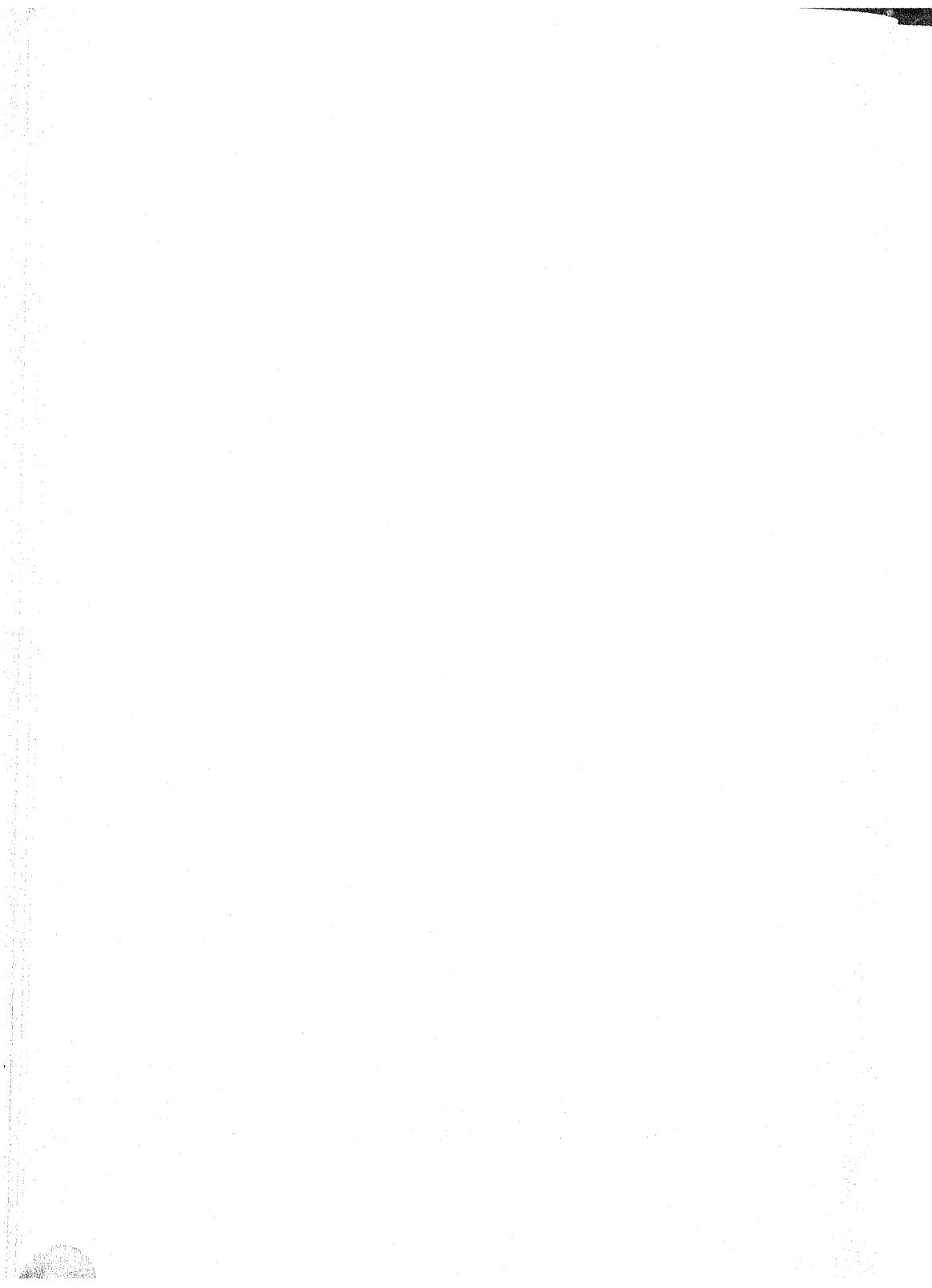
Thin-layer chromatography (as it is known today) was developed because of a specific need for a rapid method which would separate small amounts of compounds. Soon after its development it was apparent that the method was more than a micromethod for separating compounds. It was a means of (1) investigating absorbents and solvents from column work, (2) following the course of elution chromatography of colorless compounds, (3) checking the course of reactions and (4) carrying out certain reactions such as oxidations, reductions, dehydrations and so forth, directly on the strip or plate. By these means and other reactions applied directly to the unknowns, an insight into the type of compound could be gained. All these functions of thin-layer chromatography are enhanced by the speed of the method and the fact that only minute amounts of material are needed.

In general, a new technique that is developed for the separation and identification of compounds is not a method which, *per se*, displaces a previous technique. Rather it is an addition to the numerous tools available to the scientist for solving his problems.

A man that adopts the idea that a given technique is the solution to all his problems is missing an opportunity. Many times combinations of various techniques give results which surpass anything that can be attained with any of the individual techniques. Keeping this in mind it is to be hoped that this book will be an introduction to a method to those who have not used it and an aid to those who wish to further their knowledge of the subject.

J. G. KIRCHNER

Linden, N.J.



Preface

Chromatography on thin layers of adsorbent was conceived as early as 1938 and developed largely during the early 1950's. However, it has been used more extensively since about 1958 and now appears to be in an era of rapid development and almost universal adoption. At such a time, a book setting forth the basic technical details and surveying the field is in order. The author first observed the technique in the laboratory of Professor Hans Schmid in Zürich, Switzerland and was much impressed. This impression and the successful application of the method in his own laboratory have prompted the writing of this book.

The text and the bibliography cover the majority of the literature through December 1962 and a few particularly pertinent papers in 1963.

It is the intention of the author to write a condensed and non-theoretical description of this simple laboratory technique. Thus, the experimental aspects and "tricks" will be taken up, catalogued and discussed in considerable detail. In the last Chapter, an attempt will be made to tabulate, in a concise and meaningful way, as many specific applications of the method as possible. Any theoretical treatment of the subject must consist of two portions. The first is a discussion of general chromatographic theory and the second is a consideration of the deviations from the theory which arise in thin layers. Such a discussion is beyond the scope of this book.

The author would like to express his appreciation to the following persons who have made his career—and, thereby, this book—possible: Professors James L. Hall, C. L. Lazzell, L. A. Pursglove, Katherine Wilson and Elizabeth Frost Reed who are or were on the Staff of

West Virginia University; Professor Carl Djerassi of Stanford University; Professor Hans Schmid of the University of Zürich; Dr. Ulrich Weiss of the National Institutes of Health, and, finally, his parents, Mr. and Mrs. J. S. Bobbitt of Bluefield, West Virginia. Beyond doubt, the author owes his greatest debt to Professor Melville Lawrence Wolfrom of the Ohio State University and to him this book is respectfully dedicated.

The actual writing of the text would have been impossible without the assistance of the author's wife, Jane Ann Hickman Bobbitt, Drs. Roy J. Gritter, Ana Rother and R. G. Jensen of the University of Connecticut, Dr. H. K. Mangold of the Hormel Institute, University of Minnesota, and Mr. Herbert R. Simonds of the Reinhold Publishing Corporation who read portions of the manuscript at various times. The following co-workers actually carried out most of the thin-layer chromatography and assisted in the final checking and proofreading of the text: Mr. Christopher Allen, Dr. Robert Doolittle, Mrs. Judith Douville, Dr. Robert Ebermann, Dr. K. L. Khanna, Mr. Donald Kiely, Miss Judith McNew, Dr. Sardar Mahboob, Dr. Ana Rother and Mr. David W. Spiggle. The author is further indebted to his secretary Mrs. Jean Jemielity for typing the manuscript, to Mrs. Eileen Stock who made all of the drawings and to Mr. Ernest F. Spitzer and the Chas. Pfizer Co. of Groton, Connecticut for the use of their fine library.

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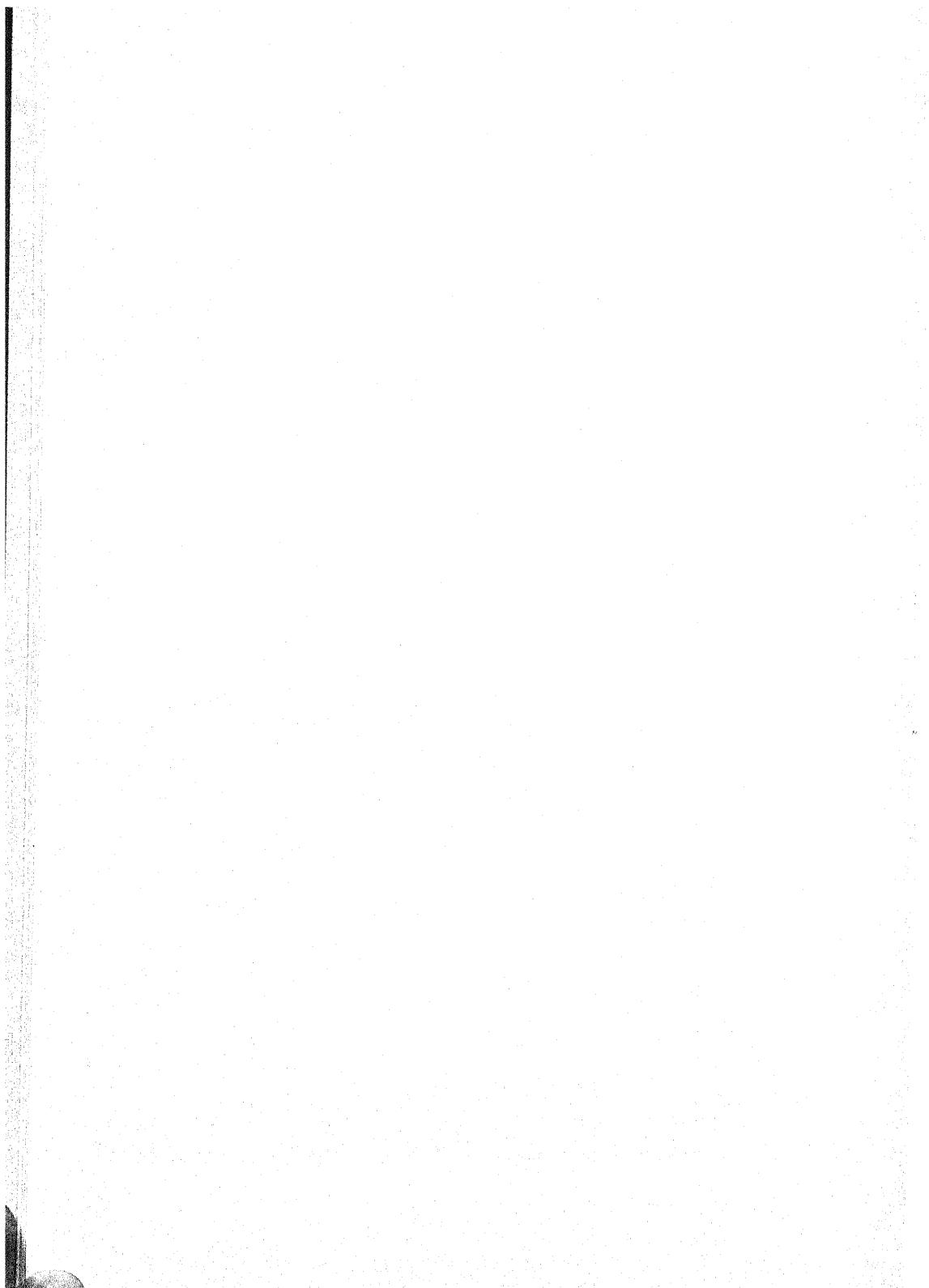
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CHAPTER 1

Introduction, History and General Applications

INTRODUCTION

Since the very beginning of the science of chemistry, its practitioners have been plagued by two technical problems. The first is to learn the purity of a given preparation or, conversely, how many components are in a given system. This information is necessary regardless of whether the system is a mineral mixture, an extract of some plant or animal, or the result of a chemical reaction. The second problem is to resolve the system into its pure components so that they can be characterized and studied. The earlier chemists performed phenomenal feats by fractional crystallization and distillation, but it has only been in the last fifty years or so that the various techniques of chromatography have promised true solutions to these problems.

It follows from the stated problems that chromatography can be considered from two viewpoints. One is *diagnostic** or qualitative and the second is *preparative.** The object of the former is to determine the number of components in a system and to learn, if possible, what they are without actually isolating them. The latter viewpoint involves the separation of a mixture into its components in such a way that reasonable amounts can be isolated and studied. Still a third aspect is *quantitative*, or concern about how much of each component is present.

* These concepts and others in this section have been well reviewed by Keulemans¹⁸⁷ and will be only briefly discussed in this volume.

All of the techniques of chromatography are based upon the same simple principle. They involve a moving system of some type (liquid or gas) which is in equilibrium with a stationary phase. These phases are so designed that the mixture to be separated will be distributed between the two. When the stationary phase is a solid and the forces acting between it and the mixture are adsorptive in nature, the technique is called *adsorption chromatography*. When the stationary phase is a simple liquid or a liquid held on some type of support, the chromatography is considered to be *partition chromatography*.

In general, adsorption chromatography involves a relatively non-polar moving phase and works best when the substances to be separated are not very polar. The major advantages over partition chromatography are that larger quantities can be separated in comparable systems and that a controlled temperature is not necessary. Partition chromatography, on the other hand, generally involves polar solvents and mixtures of very polar compounds such as carbohydrates or amino acids. Since it is basically dependent upon the distribution coefficients of the substances in question, which are, in turn, highly sensitive to temperature and other conditions, a carefully controlled atmosphere is required. The line of demarcation between adsorption and partition chromatography is, however, often blurred.

At this point, it would be appropriate to define some of the terms as they will be used throughout this book. The *adsorbent* will be the finely divided powder which makes up the stationary phase in adsorption chromatography, or holds the stationary liquid in partition chromatography. The *layer* will refer to a thin layer of absorbent, bound or unbound, deposited on a glass plate. The term *spotting* will refer to the application of the substance to be separated to the thin layer. The passing of a liquid through the layer to affect a separation will be referred to as *development* and the liquid itself will be consistently called the *developer*. The term *eluent* will be used only when the complete removal of a substance from the adsorbent is intended. The term *visualization* will refer to the rendering visible of the results of a developed chromatogram. The *Rf* will be the distance traveled by a given substance divided by the distance traveled by the solvent front. Both are measured from the origin.

Column chromatography, as originated by Tswett^{409,410} and since

practiced by legions of chemists, was primarily an adsorption technique used for preparative work. Column chromatography using an adsorbent such as silica gel or cellulose as a support for a liquid was conceived by Martin and Synge²⁴¹ and is partition chromatography for a preparative purpose. The countercurrent distribution of Craig and his co-workers^{59,60} may or may not be defined as chromatography but at least involves similar phenomena. It might also be considered a partition technique with a preparative goal. None of the above techniques have been particularly successful as diagnostic methods due to the extensive labor involved in the preparation of a single chromatogram.

The first major development in diagnostic chromatography was the paper chromatography pioneered by Consden, Gordon and Martin.^{57a,57b} The method was fantastically successful and was rapidly adopted all over the world and in every type of laboratory. The major advantages of paper chromatography are its extreme simplicity and the fact that relatively inexpensive equipment is needed. However, since it is a partition technique with the stationary liquid phase held on a piece of paper, it works best with polar developers and small amounts of polar substances. While techniques of preparative paper chromatography have been worked out and used successfully, they are laborious.

The second, primarily diagnostic technique of chromatography was gas chromatography. This was either an adsorption chromatography, as pioneered by Turner,⁴¹² Claesson⁵⁸ and Cremer,^{61,62} or a partition chromatography, as suggested by Martin and Synge²⁴¹ and introduced by James and Martin.¹⁷⁰ The advantages of gas chromatography are its speed of operation, its almost unbelievable degree of resolution and the fact that the results can be interpreted quantitatively. The disadvantages are that relatively expensive and complex equipment is needed and that the substances to be separated must have at least some vapor pressure at workable temperatures. The use of gas chromatography as a preparative technique has been quite successful although hampered by technical difficulties.

Thus, it would appear that a diagnostic technique which is adsorptive in nature and which combines the technical simplicity of paper chromatography and the speed of gas chromatography is lack-

ing. Ideally, such a method should lend itself to quantitative interpretation and should be useful also as a preparative method. Most of the conditions are fulfilled by *thin-layer chromatography*, or, as it is sometimes called, "thin-film," "open-column," "chromatostrip" or "chromatoplate" chromatography. In essence, this is a type of adsorption chromatography where the adsorbent is a thin layer of some solid deposited on a glass plate support. In operation, it is analogous to paper chromatography; that is, the substance to be separated is placed a short distance from one end of the layer and is resolved by a solvent passing through the layer by capillary action. The development is carried out in a simple closed system as in paper chromatography, but is much more rapid. When the proper solvent mixtures are used, the method can become a partition technique. Thus far, the quantitative interpretation has involved errors of 3 to 5 per cent, but the method shows promise as a preparative means for quantities of one gram or less.

HISTORY OF THIN-LAYER CHROMATOGRAPHY

It is always difficult to sort out the various originators of some concept or technique and to assign credit, because any such development is the result of contributions and ideas from many people and laboratories. Such contributions vary from the initial flash of genius to the more practical aspects such as the introduction of new or more convenient equipment or materials. The historical development of thin-layer chromatography is best divided into two such phases. The first phase is the conception and initial development of equipment and techniques followed by their slow but steady adoption. The second phase is an extensive and rapid development following the invention of new equipment, the standardization of the method and, particularly, the commercial availability of apparatus and adsorbents. A third phase will surely follow in which the method becomes a routine technique applied universally and without particular thought and attention.

In 1938, Izmailov and Shraiber¹⁶⁹ described the use of thin layers of adsorbent on glass plates for the separation of galenicals. The English summary of this paper is as follows:

"A method for chromatographic adsorption analysis is elaborated, based on the observation of the division of substances into zones on a thin layer of adsorbent, using one drop of the substance. The results obtained by the method proposed are qualitatively the same as those obtained by the usual chromatographic adsorption method of analysis. The method enables one to obtain satisfactory results using one drop of the substance under test, very small quantities of the adsorbent and minimal time. The method may be used for the evaluation of galenical preparations and their identification, as well as for a preliminary test of the adsorbent and the kind of developer."

In 1941, Crowe⁶³ described the use of thin layers of unbound adsorbent to help predict the best solvents for column chromatography. In a book entitled "Introduction to Chromatography"⁴⁴³ which was published in 1947, Williams reported the use of thin layers of adsorbent held between horizontal glass plates. The top plate had a small hole for application of the sample and the developer, and the chromatogram was made in a circular fashion.

The use of a binding agent (starch) to hold the layers in place was introduced by Meinhard and Hall²⁴⁶ in 1949. The resulting layers of alumina—"Celite" prepared on microscope slides, were used for the separation, again in a circular fashion, of inorganic salts.

The next and, by far, the most extensive and comprehensive contributions to the development of thin-layer chromatography were made by J. G. Kirchner, J. M. Miller and their co-workers at the U. S. Department of Agriculture Laboratories in Pasadena. In an extensive series of papers (1951-1957) this group investigated various adsorbents and binding agents,¹⁹³ designed equipment for the preparation of "chromatostrips"²⁵³ and used the technique for the investigation of the terpenoids.^{190-194,251-253} Narrow glass plates called "chromatostrips" (0.5 by 5.25 in.) with a silicic acid-starch layer were found to be most useful for their work although they also introduced the use of larger plates.¹⁹¹ The larger plates called "chromatoplates" by Reitsema³²³ were used by him in extensive work on essential oils.³²³⁻³²⁷

Starting a little later, but roughly paralleling the work of Kirchner and Miller, Mottier and Potterat²⁶⁸ worked out a method for analyzing food dyes on layers of nonbonded alumina and even separated amino acids by this technique.²⁶⁷ Some of the other workers in the

earlier phase (prior to 1956) were Ito, Wakamatsu and Kawahara,^{167,168} Labat and Montes,²⁰⁹ Grüner and Spaich,¹³¹ Coveney, Matthews and Pickering,⁵⁸ G. Wagner,⁴²⁶ Rigby and Bethune,³²⁸ Bryant,⁵¹ Lagoni and Wortmann,^{210,211} Demole,⁷⁶ Fukushi and Obata,¹⁰⁸ Onoe²⁸⁴ and Maruyama.²⁴² The earlier phases are well reviewed by Demole in French⁷⁷ and English.⁷⁸

The major reason for the slow development and adoption of the technique during the time from 1951 to 1958 was the fact that equipment and chemicals were not commercially available. More inertia must be overcome in the construction of equipment and the preparation of adsorbents than in signing a check or placing an order.

This last obstacle was removed by Egon Stahl in Germany. Stahl, working with C. Desaga, G. m. b. H., in Heidelberg, devised a system involving standard-size glass plates (5 by 20 cm and 20 by 20 cm), a new apparatus for the preparation of layers, and a standard adsorbent (silica gel-plaster of Paris). Subsequently, the apparatus became commercially available from Desaga and the adsorbent became available from E. Merck, A. G., in Darmstadt. Stahl and his co-workers studied the variables of the technique in a thorough manner,³⁸¹ applied it to many new types of organic compounds³⁶⁶⁻³⁸² and pioneered the use of new adsorbents. These new adsorbents immediately became commercially available. Stahl called the method "Dünnschicht-Chromatographie" or "thin-layer chromatography" ("TLC") and this name seems to be widely accepted.

The applications of the thin-layer technique mushroomed after 1958 so that it is impossible to mention all of the people who have contributed. However, some of the more prominent workers in the field have been Stahl, of course, and Kaufmann, Demole, Gänshirt, Mangold, Stanley and Seiler. The contributions of these people and many others will be discussed later.

The use of thin-layer chromatography is now almost routine in natural product, pharmaceutical and lipid laboratories, but has not been so well accepted by the organic chemists as it might. The applications to various aspects of nonlipid biochemistry have been hampered by the very polar molecules involved, but these problems are rapidly being overcome by the introduction of new adsorbents.

The field has recently been reviewed again by Demole in French⁷⁹

and English;⁸⁰ by Stahl,³⁷² Schorn³⁴⁶ and Wagner⁴²⁸ in German; by Mangold²³² and Wollish, Schmall and Hawrylyshyn⁴⁵² in English; by Michalec²⁴⁸ and Procházka³¹² in Czech; by Vioque⁴¹⁸ in Spanish; by Kokoti-Kotakis;²⁰⁵ and by Jensen.¹⁷⁶

At present, three books have been published on the subject. Two of these, one edited by Stahl³⁷⁵ with contributions from Bolliger, Brenner, Gänshirt, Mangold, Seiler and Waldi and one written by Randerath,³²⁰ are in German. The third, by Truter⁴⁰⁴ is in English.

GENERAL APPLICATIONS

The motives for the application of thin-layer chromatography are many and varied. Some of these will be considered in the following discussion. The prejudices of an organic chemist with inclinations toward natural product work will be apparent. The various points will all be fairly obvious, but may, on occasion, suggest a solution to a research problem. Examples will be cited to illustrate the discussion, although it should be noted that they are chosen at random and no attempt has been made to cite all of the possible references.

Preliminary Study of a System or Situation

The small amounts of material involved and the speed with which results are obtained make thin-layer chromatography a valuable tool for preliminary explorations of many kinds. The pharmacognosist or plant chemist can learn something about the complexity and, by the judicious choice of spray reagents, even something of the components of a plant extract. He can then use the technique to follow his gross separation procedures and to learn where the products of interest are. The same reasoning applies, of course, to various aspects of plant and animal biochemistry.

The organic chemist, or indeed, any chemist, can explore a large number of reaction conditions, using small amounts of material, in a short time. This applies to both normal synthetic reactions and attempts to degrade a natural product or unknown substance. The operator can readily learn which reactions produce new products and which yield only starting material; which reactions produce complex product mixtures and which do not. If a given material is sought and

is available for comparison, conditions can readily be found which produce it in the best yield and the cleanest form. The author and his co-workers have used this last general idea in the synthesis of the alkaloids, pilocereine³⁸ and anaferine.³³²

Intensive Study of a Single Reaction

After some idea of the best reaction conditions or best degradation scheme has been obtained for a given problem, thin-layer chromatography can be employed to learn considerably more about the reaction. A crude kinetic study of the reaction can be made by removing samples at timed intervals and chromatographing them simultaneously with starting material and the expected product, if available. This may tell whether stable intermediates are involved, whether the desired product is an end product or an intermediate and something of the course of reaction. At least, such a study will tell when all of the starting material is gone and will aid in establishing an optimum reaction time.

The author and his co-workers have used this approach extensively in the structure elucidation of the glucoside, catalposide.⁴⁰ An example from this work is shown in Figure 1.1. Others have used it in the study of the sequential methylation of phenolic acids,²²⁵ the preparation of *p*-bromophenylosazones of xylose derivatives,⁷ the glycerolysis of linseed oil³³⁵ and the biogenetic production of terpenes in mint plants.²⁰ The presence of an unstable intermediate was shown in the synthesis of pseudoionone.²³⁷

The Isolation of Reaction Products

Thin-layer techniques can be applied to the isolation of reaction products in several ways. First of all, if acid-base partition, distillation or crystallization methods are to be used, one can easily learn something of the precision and completeness of the separations and the purity of the various fractions. Furthermore, the short times involved in learning where the product is will satisfy even the most impatient chemist.

If simple separation methods fail and the products must be separated by chromatography, thin layers offer several possibilities. Quantities less than one gram can be separated by preparative thin-

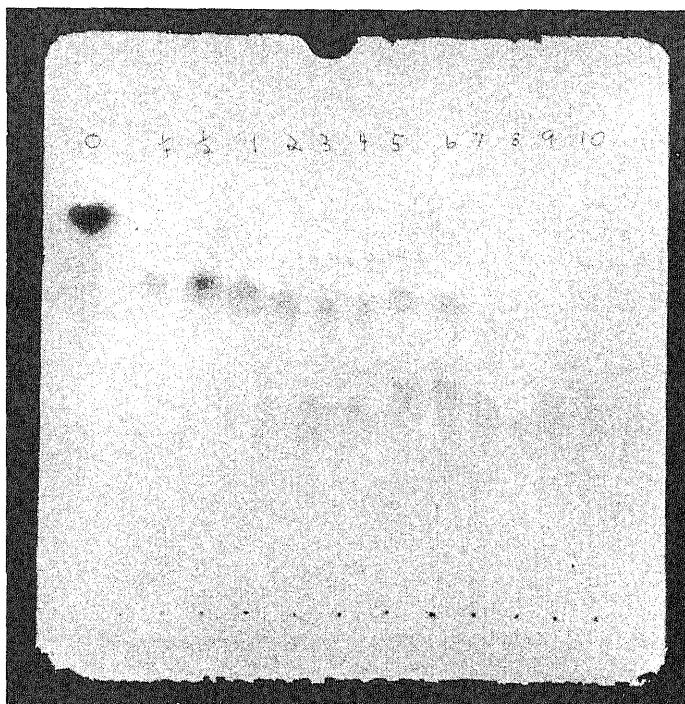


Figure 1.1. Kinetic study of the saponification and epoxide opening of the glucoside catalposide.⁴⁰ The starting material shown on the left at zero time is converted after one hour to a deshydroxybenzoyl derivative (middle spots) which is, in time, converted to a glycol (lower spots). The example clearly shows the presence of an intermediate and indicates the optimum reaction time for the preparation of either product. (Photograph by S. E. Wollman)

layer chromatography. This is, beyond doubt, easier, quicker and more precise than column chromatography and is discussed extensively in Chapter 9. In the author's laboratory, this has proved to be an ideal method for purifying samples prior to a final crystallization for microanalysis. Specific examples of this are noted in the tables in Chapter 11.

Applications to Column Chromatography

The simplest type of column chromatography involves the removal of small amounts of contaminants or tars by passing the sam-

ple, in a suitable solvent, over a short column of adsorbent. Such a process is adaptable to relatively large quantities and gives high yields. Thin-layer techniques can predict, in a short time, the ideal solvent and adsorbent. One needs only to find a system in which the product moves and the contaminant stays at the origin and to transpose this to a short column of the *identical* adsorbent. Normally, the adsorbents used for thin layers are of such small particle size that a pressure system or a vacuum system must be used when they are placed in columns. Such a scheme has been used by Kirchner and Miller¹⁹⁰ for the preparation of terpeneless essential oils. The reverse of this notion, that is, the isolation of small amounts of product from mixtures, has been used to isolate insecticide residues from plant material.

If the quantities involved and the degree of separation required are such that normal column chromatography cannot be avoided, thin-layer techniques can be used to predict the best solvent system and, above all, to analyze the effluent from the column. Several authors have stated or implied^{90,251,365} that the solvents used to give good separations on thin layers can be used directly on a column of the same adsorbent. Duncan⁹⁰ has published the following formula for predicting whether two substances will separate on a column.

$$r = \frac{a}{b + 0.1a}$$

$a = Rf$ of faster moving substance on a thin layer

$b = Rf$ of slower moving substance on a thin layer

Separation can only be expected under the conditions prescribed by Duncan (very high adsorbent-material ratios) when r is greater than unity.

It should be pointed out, however, that all of these situations depend upon the actual magnitude of the thin-layer Rf values. This is particularly true in respect to the Duncan expression which would certainly not be valid when the substances run close to the solvent front. The separations on a column will depend primarily on the retention times of the substances involved and these retention times, as stated for thin-layer chromatography by Zoellner and Wolfram,⁴⁵⁸ are inversely proportional to the Rf values of the substances on the same

adsorbent. Thus, a small difference when R_f values are small can be expected to produce an acceptable difference in retention times and a separation, whereas a large difference when R_f values are close to unity will not give a separation. The author has had some success using the following scheme. A pair of solvents is found which will produce an acceptable separation on a thin layer. The polar component of the solvent system is then reduced until the substances to be separated show R_f 's of about 0.2. This modified system is then used directly on a column of adsorbent identical in every way with that in the thin layer.

The monitoring of the effluent of a column is normally done by analyzing fractions obtained using a fraction cutting device. On a typical large layer (20 by 20 cm), as many as eighteen samples can be analyzed simultaneously. The fractions are then combined according to their components. Such applications are also noted in the tables in Chapter 11, and an example taken from the author's work on catalposide is given in Figure 1.2.

Routine Quantitative Assay

A number of procedures have been published for quantitative assay by thin-layer chromatography. These are discussed in Chapter 10 and documented in Chapter 11. Most of the work has been done in clinical chemistry where speed is an important factor. The major limitation of the technique is the error of about 3 to 5%.

Thin-Layer Chromatography in Conjunction with other Methods

The use of thin-layer chromatography in conjunction with gas chromatography has been especially useful in the lipid field.^{178,232,233,236} Normally, the class separations are made preparatively on thin layers and the classes are resolved, generally after chemical modification, by gas chromatography. In this manner, it is possible to take advantage of the ease of quantification and the high degree of resolution of the latter method. It should be noted, however, that the classes are also often resolved by "reversed phase" thin-layer chromatography.^{181,182,184,230,232 and others}

A method has been suggested¹⁴⁷ for the combined use of thin-layer chromatography and the mass spectrograph. The silica gel taken

2.90 g. of mixture on 180 g. of Silica Gel G

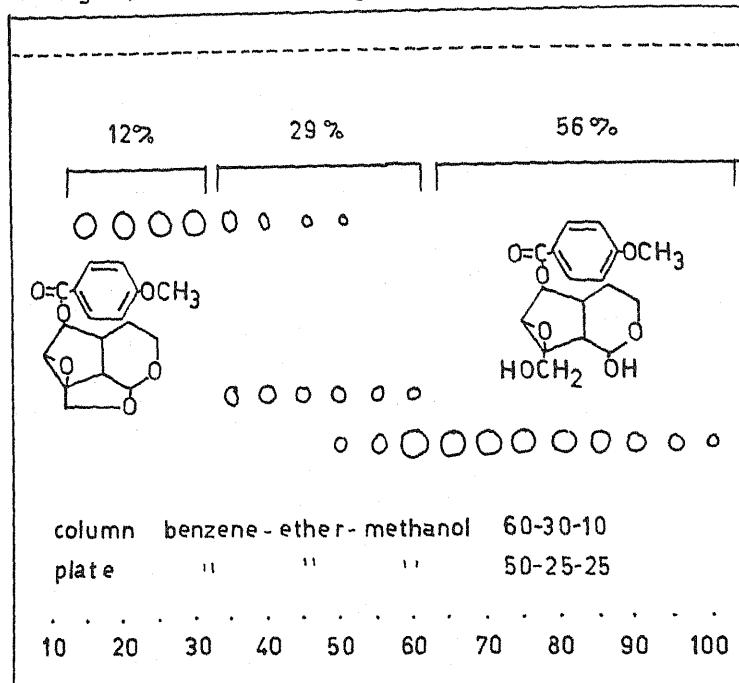


Figure 1.2. A thin-layer analysis of the chromatographic separation of two derivatives of catalposide.¹⁰ The eluent from a Silica Gel G column was divided using an automatic fraction collector and every fifth tube was examined. The solvents for the column were predicted from thin-layer data as described in the text. The solvent systems used on the column and the analysis layer as well as the structure of the materials isolated are shown. The experiment clearly shows the degree of separation, the relatively short time involved (first sample came off after only fifteen 20 ml fractions) and which fractions contain pure compounds. The center spots represent an, as yet, unknown compound.

from the layer and containing the substance to be investigated is introduced directly into the instrument. The adsorbent does not appear to have any effect on the spectrum. The ratio of sample to adsorbent must be at least 1:100, however, and several micrograms of substance are needed.

The Use of Thin-Layer Patterns

Thin-layer chromatography, like paper chromatography, can be used to determine characteristic component-patterns for drugs, plant

extracts and biochemical preparations. The comparison of these with patterns of adulterated or diseased samples can provide interesting results. Such techniques have been used to study "chemical races" in plants,⁴⁵³ drug contaminants,¹⁶¹ mint oils³²³ and lipid components of multiple sclerosis patients.¹⁵⁷

Classification Systems

In the alkaloids,⁴³⁶ the estrogens²²¹ and mint oil constituents,³²³ thin-layer chromatographic properties have been correlated to some extent with structural types, and classification procedures have been developed. Such systems make it easier to identify known compounds and to obtain information about unknown ones.

CHAPTER 2

Adsorbents

INTRODUCTION

The adsorbents most commonly used in thin-layer chromatography are, in the following order, silicic acid or silica gel,* aluminum oxide or alumina, kieselguhr or diatomaceous earth and powdered cellulose. Those which have been used to a lesser extent are polyamide powders, ion-exchange powders (modified cellulose), "Florisil," calcium sulfate, polyethylene, "Magnesol," hydroxylapatite, "Sephadex," zinc carbonate and various mixtures of these. Kirchner, Miller and Keller¹⁹³ explored several adsorbents using starch and plaster of Paris as binding agents. These data are shown in Table 2.1. Although they pertain only to the separation of essential oils by adsorption processes, they show the large number of substances which can be formed into usable layers. Only calcium hydroxide layers are really poor and, as will be discussed later, the addition of silica gel produces usable layers of this substance. Essentially any substance which has desirable adsorptive and chemical characteristics can be successfully used in thin-layer chromatography.

The four most widely used adsorbents, *silica gel*, *alumina*, *kieselguhr* and *cellulose*, however, offer a wide range of properties and can be used in most cases. Waldi, Schnackerz and Munter⁴³⁶ made a comparative study of Silica Gel G, Aluminum Oxide G and Kieselguhr G with the following conclusions. Silica gel and alumina are, respectively, essentially acidic and basic in character while kieselguhr is

* The major difference between silicic acid and silica gel appears to be solely in the method of preparation. They are chemically identical, but the gel is prepared in such a manner as to enhance its adsorptive power.

TABLE 2.1. CHARACTERISTICS OF CHROMATOSTRIPS MADE
WITH VARIOUS ADSORBENTS^{193*}

| Adsorbent Coating | Physical Characteristics of Strip | Resolution of Essential Oils |
|------------------------|--------------------------------------|------------------------------------|
| Magnesium oxide | soft | none |
| Alumina | excellent | good |
| Alumina + silicic acid | excellent | good |
| Calcium hydroxide | soft, crumbly | none |
| Starch | good | none |
| Dicalcium phosphate | fair | some resolution |
| Bentonite | good | oils decomposed |
| Calcium carbonate | good | slight resolution |
| Magnesium carbonate | fair | slight resolution |
| "Filtrol" | good | separation, but oils decomposed |
| "Filtrol" X202 | good | separation, but oils decomposed |
| "Filtrol," neutral E | good | separation, but oils decomposed |
| "Florisil" | good | fair separation |
| Talc | good | slight separation |
| Silicic acid | excellent | excellent |

* Reproduced from Kirchner, Miller and Keller, *Anal. Chem.*, 23, 420 (1951) through the courtesy of the authors and The American Chemical Society.

neutral. Furthermore, silica gel has the highest capacity (the ability to separate the largest quantity of a mixture) followed by alumina and kieselguhr in that order.

Alumina chromatography is essentially adsorption chromatography; silica gel can function in both adsorption and partition chromatography depending upon the solvent system and kieselguhr is best characterized as a support for partition phenomena. Thus, alumina would be the adsorbent of choice for the separation of non-polar basic or neutral mixtures. Silica gel can be used for nonpolar acidic mixtures and, because of its higher capacity, would even be preferred for the nonpolar neutral materials. Silica gel, kieselguhr and cellulose are all suitable for the separation, through partition processes, of polar molecules. Of course, the properties of these adsorbents can be extensively altered by addition of various acids, bases and buffers so that many purposes can be served which are not covered by the above generalizations.

Although artifact formation on thin layers is rare, it is not unknown. Steroid 16- β -esters have been shown to undergo reactions on alumina layers²⁴⁷ and ethylene ketals have been hydrolyzed on silica gel layers.²⁶³ Alumina in columns is known to catalyze ester hydrolysis, isomerization of double bonds and other reactions.²³² The chromatography of sugars on silica gel layers with ammonia solutions has been shown⁴³⁹ to produce amination reactions.

The various adsorbents can be and are sometimes used in a pure form, but more frequently, they are used in combination with small amounts of added components. These additives can be blended with the solid adsorbent before it is made into layers, or they can be dissolved in the water portion of the slurry which, in most cases, is the vehicle for application to plates. In general the following purposes are served by the additives.

(1) They may serve as binders or substances to make the adsorbent more cohesive and to hold it to the plate. These make the layers much easier to handle and use and make it possible for them to be sprayed with visualizing reagents. The most common binder is plaster of Paris (calcined calcium sulfate, $\text{CaSO}_4 \cdot \frac{1}{2}\text{H}_2\text{O}$) which is added to the dry adsorbent in amounts up to 20 per cent. The first binder used by Meinhard and Hall²⁴⁶ and by Kirchner and his group¹⁹³ was starch, which, although it gives the layers an added stability over plaster of Paris, limits the spray reagents which can be used to visualize the finished chromatograms. Other binders which have been used are polyvinyl alcohol,²⁸⁴ collodion³¹⁷ and an alcohol-soluble polyamide, "Zytel 61."^{*153} Two binders which have been added at later stages are decalin,²⁵⁴ which is added to the developing system and dichlorodimethylsilane,¹⁸⁵ which is applied to the developed plates so that they can be washed like paper chromatograms. The relative advantages and disadvantages of these binders will be discussed in more detail in the section of this chapter devoted to silica gel.

(2) The additives may serve to alter the properties, both physical and chemical, of the adsorbents. Thus, acids, bases and buffers are commonly incorporated into the layers. Reagents which will complex or chelate to a greater or lesser extent with the substances to be sep-

* Du Pont de Nemours, Geneva, Switzerland

arated, are often added. The latter concept is a relatively recent one and will, in the author's opinion, become important.

The impregnation of layers with hydrophobic liquids so that "reversed phase" chromatography can be carried out will be discussed in Chapter 3.

(3) On occasion, materials are added to adsorbents as "fillers." These reduce the activity of the adsorbent and make it somewhat more porous, resulting in shorter developing times. This concept was originally used by Meinhard and Hall²⁴⁶ who added "Celite" (see p. 24) to starch-bound alumina. More recently, Bennett and Heftmann^{26,27} have added Kieselguhr G to Silica Gel G for a similar purpose.

(4) Finally, reagents may be added which will aid in the visualization of the completed chromatogram. The incorporation of phosphors or fluorescent compounds in the adsorbent so that unsaturated compounds can be seen or easily rendered visible in ultraviolet light is a common practice. On occasion, sulfuric acid is added to the adsorbent. This alters the character of the adsorbent and allows the visualization of the completed chromatogram by heating (to char the organic compounds).

Procedures have been developed by Stahl³⁷³ and by Heřmánek, Schwarz and Čekan¹⁴⁴ for relating the activity of alumina layers to the Brockmann⁵⁰ activity scale.

A number of these adsorbents, both modified and pure, are commercially available. These sources will be listed in Table 2.4 at the end of this chapter, although, at the appropriate places, procedures will be given so that an investigator can prepare his own.

Silica Gel or Silicic Acid

Silica gel, used with a plaster of Paris binder is, by far, the most extensively used adsorbent in thin-layer chromatography. It forms a versatile layer which can serve as the solid stationary phase for adsorption chromatography, as a support for the polar liquid phase in normal partition chromatography and as a support for the nonpolar liquid phase in "reversed phase" chromatography. The distinction between the adsorption and normal partition chromatography in this case lies in the solvent system chosen for the chromatogram. If

the system consists of solvents such as hexane, benzene, ether, methanol or similar substances, adsorption phenomena will result. If a system is chosen which contains both a nonpolar liquid and a polar liquid such as water, the result will probably be partition chromatography. In "reversed phase" chromatography, the silica gel layer is impregnated with a nonpolar liquid such as a Silicone oil or a hydrocarbon before use. This will be discussed in Chapter 3.

Two binders, starch and plaster of Paris, are commonly used with silica gel. Both originated with Kirchner, Miller and Keller.¹⁹³ Starch gives a more mechanically stable layer^{8,120,193} which can actually be written on with a blunt lead pencil, but the inclusion of starch in the layer forbids the use of corrosive spray reagents for visualizing the finished chromatogram. (Cold concentrated sulfuric acid may be sprayed on the layers without difficulty, but the sprayed layers cannot be heated to char the spots.) The use of these corrosive spray reagents (sulfuric acid, chromic acid, nitric acid, etc.) as essentially universal reagents for organic substances, is one of the greatest advantages of thin-layer chromatography. On the other hand, the presence of calcium ion (from the plaster of Paris) in the layers has caused some difficulty in the chromatography of inorganic ions³⁵⁵ and nucleotides.³¹⁷

Silica Gel with No Binder. Silica gel has been used only rarely^{1,51,312,322} without some sort of binder. This situation will surely change, however, due to the recent introduction of Woelm "Silica Gel for Thin-layer Chromatography," which contains no binder and depends, for the stability of its layers, on a small particle size. The commercial preparation "Anisil" has no binder as such, but does contain 10 to 15 per cent of magnesium oxide which enhances its layer stability and makes it a basic adsorbent.

A method for the preparation of a silica gel suitable for thin-layer chromatography has been given by Adamec, Matis and Galvánek.¹ The starting material was water glass (mixed, hydrated sodium silicates) and the adsorbent was used without a binder.

Silica Gel with a Plaster of Paris Binder. The commercial preparation Silica Gel G (G for *gips* or gypsum) is of this type. It is manufactured by Merck in Germany according to the specifications of

Stahl and, without doubt, is the most widely used adsorbent in thin-layer chromatography. Mangold²³² has stated that a reasonable approximation of the commercial product can be prepared by thoroughly mixing Mallinckrodt Silicic Acid, 200 mesh, with 10 to 15 per cent of newly calcinated calcium sulfate (prepared by heating reagent grade $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ at 180° for 24 to 48 hours²⁹⁶) of the same grain size. A 200-mesh screen produces particles smaller than 0.12 mm.

Silica Gel G contains appreciable amounts of ferric ion. This does not interfere with the separations of organic compounds, but must be removed for successful work with inorganic ions.³⁵⁵⁻³⁶⁰ Seiler and Rothweiler³⁵⁸ give the following instructions (freely translated) for doing this.

"Five hundred grams of Silica Gel G is treated with 1000 ml of 6*N* hydrochloric acid (500 ml of concentrated hydrochloric acid and 500 ml of distilled water), stirred and allowed to stand. The supernatant liquid, colored yellow by the iron, is decanted and the silica gel is washed twice more with acid and then with three successive 1000-ml portions of distilled water. The adsorbent is then filtered and washed with distilled water until the filtrate is only slightly acidic. It is finally washed with 250 ml of ethanol and 250 ml of benzene and dried in an oven at 120° ."

The material from the above treatment *now contains insufficient binder*. Two grams of plaster of Paris³⁵⁹ or one gram of starch³⁵⁸ are added to 28 g of purified silica gel to obtain a usable adsorbent.

When materials are to be recovered from thin-layer adsorbents by elution, it is suggested^{244,394} that the adsorbent be prewashed. This can be done for Silica Gel G by extracting it three times with boiling methanol²⁴⁴ or by allowing it to stand overnight with the same solvent.³⁹⁴ Honegger found¹⁵⁸ that 50 g of Silica Gel G yielded the following amounts of impurity on elution: with chloroform, 6.8 mg; with benzene, 5.5 mg and with acetone, 10.5 mg. Or, the layers can be prewashed with the developing solvent.

Silica Gel With a Starch Binder. To the author's knowledge, no commercial preparation of this type is available. Kirchner, Miller and Keller¹⁹³ combine 19 g of Merck reagent grade silicic acid, 100

mesh, one gram of "Clinco"^{*} starch, ** 0.15 g of zinc silicate and 0.15 g of zinc cadmium sulfide. The latter two reagents are added as phosphors, but the same proportions would surely serve for the preparation of layers without them. The preparation of starch bound layers is inherently different from the preparation of plaster of Paris bound layers in that the slurry of adsorbent, starch and water is heated before it is applied to plates. This will be discussed in Chapter 3.

Silica Gel with Fluorescing Agents. Several reagents are commonly incorporated into thin layers so that the resulting spots on the developed chromatogram can be seen without using a spray reagent. This is particularly helpful in preparative work. The fluorescing agents, with one exception, are usually blended with the dry adsorbent before the layers are prepared. The exception is sodium fluorescein which is normally dissolved in the liquid used to make the slurry. When the developed plates are placed in short wave ultraviolet light, compounds containing conjugated double bonds show up as dark spots. The various fluorescent materials, the amounts used and their commercial sources are given in Table 2.2.

Acidic Silica Gel. Acids have been incorporated into thin layers for two reasons. The first is to modify the properties of the adsorbent and the second is to aid in visualization. Thus, Stahl³⁶⁹ used 0.5N aqueous oxalic acid rather than pure water to prepare layers. The adsorption activity of such plates is slightly increased.

In contrast to this, Reichelt and Pitra³²² deactivated silica gel by adding 25 per cent water or 43 per cent of dilute (50 per cent) acetic acid to the dry activated powder. In this case, the silica gel was then used without a binder for the separation of cardenolides.

Layers prepared from adsorbents containing small amounts of sulfuric acid can easily be visualized by heating the developed chromatograms on a hot plate or in an oven to char the organic substances. Peifer²⁹⁶ added 2.5 ml of concentrated sulfuric acid to 100 ml of chloroform-methanol, (70:30) v/v, which was subsequently slurried

* Clinton Foods, Inc., Clinton, Iowa.

** Dr. Kirchner has informed the author that still another starch binder is made from ordinary corn starch and "Superior AA Tapioca Flour" mixed in a ratio of 2 to 1. The tapioca flour is a product of Stein, Hall and Co., Inc. of New York. Furthermore, the amount of starch can conveniently be cut in half from the original proportions.

TABLE 2.2. FLUORESCENT AND PHOSPHORESCENT CHEMICALS USED
IN THIN-LAYER CHROMATOGRAPHY

| Name | Amount Used | Commercial Source | Ref. |
|---|----------------------------|---|------|
| 1. Zinc silicate luminescent material P 1, Type 118-2-7 | 2% in adsorbent | General Electric Co., Cleveland, Ohio | 46 |
| 2. Rhodamine 6G | 0.003% in adsorbent | Matheson, Coleman and Bell, East Rutherford, N.J. | 323 |
| 3. Luminescent substance ZS-Super | 2% in adsorbent | Riedel-de Haen, 3016 Seelze bei Hannover, Germany | 117 |
| 4. "Ultraphor" | 0.005% in adsorbent | Badische Anilin and Soda Fabrik, Ludwigshafen (Rhein), Germany | 293 |
| 5. Luminescent chemical No. 601 | 1% in adsorbent | E. I. du Pont de Nemours & Co., Photo Products Dept., Wilmington 98, Delaware | 232 |
| 6. "Sodium fluorescein" | 0.04% sol. in water phase | | 366 |
| 7. Zinc silicate-zinc cadmium sulfide | 0.75% of each in adsorbent | Du Pont phosphors No. 601 and No. 1502 respectively | 193 |

with 50 g of Silica Gel G. The developed chromatograms (in this case on microscope or lantern slides) were visualized by heating.

Basic Silica Gel. Stahl³⁶⁹ described the preparation of basic layers using 0.5*N* aqueous potassium hydroxide rather than water to make the slurry. The layers were found to be somewhat less active than normal. Such plates can be used to separate acids, which stay at the origin, from neutral compounds or bases which move with the solvent. Adsorbents consisting of 80 per cent calcium hydroxide and 20 per cent Silica Gel G⁴⁴⁹ will be more properly considered as a separate adsorbent system. The commercial material "Anisil," as noted above, is a basic adsorbent.

Buffered Silica Gel. In general, buffered silica gel layers have been used for the separation of polar or ionic compounds by partition chromatography. The addition of the buffer is normally made in the same manner as with acids and bases, that is, by using the desired buffer solution in place of water to make the slurry. Such slurries (at least those made with Silica Gel G) must be spread quickly since they thicken more rapidly than usual.²³² The layers should be allowed to stand several hours at room temperature before they are oven-dried.²³²

TABLE 2.3. THE USE OF BUFFERED LAYERS IN THIN-LAYER CHROMATOGRAPHY

| Buffer Sol. | Concentration | Compounds Separated | Ref. |
|---|---------------------------|---|----------------------|
| 1. Sodium acetate | 0.3 m 0.02 m | flavones sugars | 379, 380 307, 377 |
| 2. Monopotassium phosphate and disodium phosphate | equal amounts of 0.2 M | amino acids | 271 |
| 3. Citric acid-phosphate buffers | pH 7 | sugars | 307 |
| 4. Boric acid | 0.1 N | sugars | 307 |
| 5. Ammonium sulfate | 10% | acidic lipids and amphoteric phospholipids | 234 |

This helps to prevent flaking which is also more pronounced with buffered layers. Some of the uses of buffered layers will be summarized in Table 2.3.

The use of boric acid in the separations of sugars is interesting because of the formation of borate complexes between adjacent *cis*-hydroxyl groups. Such complexes are probably responsible for the effectiveness of the buffer.

The use of buffered layers for thin-layer ionophoresis by Honegger¹⁵⁶ is a rather special case since such solutions are normally used to carry current and to form complex ions which can be caused to migrate. Honegger used 0.1 M sodium citrate and acetic acid-formic acid solutions.

Silica Gel Containing Complexing Agents. The addition of complexing agents to thin layers to increase the power of resolution is an intriguing notion and will surely be further developed. At present, the only clear case of this is the use of silver nitrate on silica gel by Barrett, Dallas and Padley¹⁶ and by Morris²⁶⁰ to separate certain closely related lipids. The basis of this separation is the tendency of silver ion to complex with the pi electrons of double bonds to a greater or lesser degree. The former authors used 12.5 per cent silver nitrate to make a slurry with Silica Gel G. A similar result was produced by Morris²⁶⁰ who sprayed layers with a saturated silver nitrate solution or a saturated methanolic boric acid solution (see p. 48) or both.

As previously stated, the use of boric acid layers for sugar separation may well be an example of complex formation. The use of 2,5-hexanedione in the moving phase for the separation of inorganic ions³⁵⁹ is an example of the application of this notion in a reverse manner.

Alumina

Historically, alumina was the first adsorbent used for thin-layer work. Izmailov and Shraiber,¹⁶⁹ Meinhard and Hall²⁴⁶ and the Kirchner group¹⁹³ all used or explored this adsorbent. It is strange, then, that alumina has not been more extensively used than it has. One possible reason for this is that alumina is a more reactive adsorbent than silica gel and is known to catalyze ester hydrolysis, isomerization of double bonds, and other reactions.²³² Also, alumina is a basic substance⁴³⁶ in contrast to the acidic silica gel and has a somewhat lower capacity.⁴³⁶ Thus, one would tend to choose it for the separation of basic or neutral substances only when silica gel separations were poor.

Alumina with No Binder. Again, in contrast with silica gel, alumina has been used to a large extent with no binder. The reasons for this are obscure but probably can be traced to the ready availability of chromatographic grades of alumina and the large body of knowledge which has accumulated on alumina chromatography. Furthermore, layers prepared by simply leveling dry alumina powder on plates give quite good separations. Thus, no slurries or elaborate application apparatus are needed. These layers are mechanically unstable and must be sprayed with caution.⁷⁰

The alumina which is used in this manner is normal, commercially available alumina of any desired activity grade having a particle size of 0.1 mm or 200 to 300 mesh. Recently, Woelm has announced a special "Alumina for Thin-layer Chromatography" which is available in acid, basic and neutral grades and which is more cohesive than usual. This enhanced cohesiveness is produced by smaller particle size.

Huneck¹⁶⁵ has published directions for the preparation of a fibrous alumina which is quite cohesive and which forms stable layers with no binder.

Alumina with a Plaster of Paris Binder. This adsorbent is com-

Cellulose without a Binder. The fibrous nature of powdered cellulose permits it to be shaped into stable layers without a binder.^{321,450}

Cellulose with a Plaster of Paris Binder. The commercial product, Cellulose Powder MN 300 G, is of this type. Randerath³¹⁹ has systematically compared this material with paper for the separation of various nucleic acid derivatives. He found that the cellulose layers gave smaller, more compact spots and better separations in a shorter time (90 min) than paper (6 to 8 hr). Wollenweber⁴⁵¹ found similar results with food dyes.

Cellulose Ion-Exchange Powders. Two cellulose powders, modified to produce ion-exchange resins, have been used for the separation of nucleotides.^{316-318,320} These are DEAE-cellulose (diethylaminoethyl cellulose) and ECTEOLA-cellulose (epichlohydrin linking triethanolamine with cellulose). They have been used with a collodion binder^{316,317} or without a binder.³¹⁸

A large number of cellulose powders, plain and modified, bound (G series) and unbound, are commercially available (Table 2.4). In fact, several are available which have not yet been mentioned in the literature.

Ion-Exchange Resins

Berger, Meyniel and Petit²⁹ have reported the use of "Dowex" 1 and "Dowex" 50 (both anion and cation exchangers, 200 to 400 mesh) for the separation of organic and inorganic compounds. They used a 1:1 mixture of the resin and commercial plaster of Paris-bound cellulose, Cellulose Powder MN 300 G.

Polyamide Powders

Polyamide powders are normally used without binders and have been developed by two groups. Davídek and his co-workers^{69,71,74} have spread commercially available polyamide powder on plates and used the resulting layers for the separation of such polar molecules as flavonoids and gallic acid esters. Wang and his co-workers^{219a,436a} allowed 20 per cent solutions of ϵ -polycaprolactam resin in 80 per cent formic acid to evaporate on glass plates and used the resulting thin layers for the separation of phenols.

A commercial product is now available from Woelm (see Table 2.4).

Hydroxyl-Apatite

Hofmann has reported the use of hydroxyl-apatite (a complex calcium phosphate hydroxide*) for the separation of 1- and 2-mono-glycerides¹⁵⁵ and of proteins.¹⁵⁸ In the former case, he used plaster of Paris as a binder and in the latter case, "Zytel 61" (see p. 16).

"Celite"

"Celite" No. 545 with a plaster of Paris binder has been used¹¹⁴ for the separation, by partition processes, of steroids. The layers were impregnated with formamide. Such layers have extremely short developing times (3 to 7 minutes).

"Sephadex"

"Sephadex" G 25, a cross-linked dextran which differentiates according to the molecular weight of the molecules being separated, has been used for the chromatography of proteins.⁸² A modified adsorbent, "DEAE-Sephadex" has been used for the separation, by gradient elution, of proteins and nucleotides.⁸²

Polyacrylonitrile "Perlon" Mixtures

Mixtures of polyacrylonitrile and "Perlon" (a polycaprolactam powder) have been used, in a buffered state, to separate anthocyanins and such very polar molecules as sugars.³⁶

Zinc Carbonate

Zinc carbonate with 5 per cent starch as binder has been used for the separation of 2,4-dinitrophenylhydrazones.⁹

Polyethylene Powder

Polyethylene powder, "Hostalen S," has been used²³² for the separation of fatty acids and their methyl esters.

Calcium Sulfate as Adsorbent

Layers prepared from pure calcium sulfate have been used for the separation of steroids²⁴³ and lipids.¹⁸⁰ The major advantage of such

* Prepared according to Anacker and Stoy^{4b}

plates is that they can be mechanically washed like paper chromatograms. Thus, even more spray reagent systems can be used.

"Magnesol" (Adsorptive Magnesium Silicate)

This adsorbent with a starch binder was preferred by Bryant⁵¹ and by Pryor and Bryant³¹³ for the separation of the essential oils. A commercial product containing no binder but especially prepared for thin-layer work is available.

Calcium Hydroxide with Silica Gel G

Layers prepared from a mixture of calcium hydroxide and Silica Gel G (6:1) and (4:1) have been found to produce good separations in the carotenoid field.^{166,449}

"Florisil" (Mixed Silica Gel and Magnesia)

Peifer²⁹⁶ has described a procedure for the preparation of micro-chromatoplates from "Florisil"-plaster of Paris (see p. 46).

CONCLUSION

Obviously, any finely divided material having the desired adsorptive, structural or chemical properties can be used as an adsorbent for thin-layer chromatography. Probably, the area which will be developed most fruitfully is the use of mixed adsorbents. Several of these are given above, and others, such as Silica Gel G-Kieselguhr G mixtures, have been reported.^{26,27}

It is rather interesting that the one factor which delayed the development of this technique, that is, commercial availability of materials, has now progressed so far that several adsorbents are available (the various types of cellulose) for which no published uses are recorded.

The commercial sources of most of the adsorbents are given in Table 2.4 and the specific adsorbents which have been used for specific separations are detailed in Chapter 11.

Thin-Layer Chromatography

TABLE 2.4. COMMERCIAL SOURCES OF THIN-LAYER CHROMATOGRAPHY ADSORBENTS

| Commercial Name | Nature | Manufacturer | U. S. Source |
|--|--|--|--|
| "Anisil", Silica Gel Woelm | silica gel-magnesium oxide pure silica gel | Analytical Co., Hamden, Conn. M. Woch, Eschwege, Germany | Alupharan Chemicals, 616 Commercial Pl., P.O. Box 755, New Orleans, La. |
| Silica Gel G | silica gel with plaster of Paris | E. Merck, A. G., Darmstadt, Germany | Brinkmann Instruments, 115 Cuttermill Rd., Great Neck, N.Y. |
| Silica Gel H Silica Gel HF | pure silica gel pure silica gel with inorganic fluorescent material | E. Merck E. Merck | Brinkmann Instr. Brinkmann Instr. |
| Silica Gel GF | silica gel with plaster of Paris and in- organic fluorescent material | E. Merck | Brinkmann Instr. |
| Silica Gel D5 | silica gel with 5% plaster of Paris | Fluka, A. G., Buchs, Switzerland | Gallard - Schlesinger, Chemical Mfg. Corp., 1001 Franklin Ave., Garden City, N.Y. |
| Aluminum Oxide DO Alumina Woelm Basic Alumina Woelm Neutral Alumina Woelm Acidic Aluminum Oxide G Aluminum Oxide D5 Aluminum Oxide D5F | pure aluminum oxide pure basic aluminum oxide ¹ pure neutral aluminum oxide ¹ pure acidic aluminum oxide ¹ aluminum oxide with plaster of Paris aluminum oxide with 5% plaster of Paris aluminum oxide with 5% plaster of Paris and a fluorescence indicator | Fluka Woelm Woelm Woelm E. Merck Fluka Fluka | Gallard-Schlesinger Alupharan Alupharan Alupharan Brinkmann Instr. Gallard-Schlesinger Gallard-Schlesinger |
| Kieselgeln G Cellulose Powder MN 300 | diatomaceous earth with plaster of Paris pure cellulose powder | E. Merck Macherey Nagel & Co. | Brinkmann Instr. Brinkmann Instr. |
| Cellulose Powder MN 300G Cellulose Powder MN 300 F254 | cellulose powder with plaster of Paris | Macherey, Nagel & Co. | Brinkmann Instr. |
| Cellulose Powder MN 300 GF254 Cellulose Powder MN 300/AC | cellulose powder with an inorganic fluores- cent material cellulose powder with plaster of Paris and an inorganic fluorescent material pure acetylated (10%) cellulose | Macherey, Nagel & Co. | Brinkmann Instr. |

| | | | |
|--|--|---|------------------|
| Cellulose Powder MN 300 G/AC | acetylated (10%) cellulose with plaster of Paris | Macherey, Nagel & Co. | Brinkmann Instr. |
| Cellulose Powder MN 300 CM | pure carboxymethyl cellulose Paris | Macherey, Nagel & Co. | Brinkmann Instr. |
| Cellulose Powder MN 300 G/CM | carboxymethyl cellulose with plaster of Paris | Macherey, Nagel & Co. | Brinkmann Instr. |
| Cellulose Powder MN 300 P Cellulose Powder MN 300 | pure cellulose phosphate with plaster of Paris | Macherey, Nagel & Co. | Brinkmann Instr. |
| G/P | cellulose phosphate with plaster of Paris | Macherey, Nagel & Co. | Brinkmann Instr. |
| Cellulose Powder MN 300 DEAE | pure diethylaminoethyl cellulose | Macherey, Nagel & Co. | Brinkmann Instr. |
| Cellulose Powder MN 300 G/DEAE | diethylaminoethyl cellulose with plaster of Paris | Macherey, Nagel & Co. | Brinkmann Instr. |
| Cellulose Powder MN 300 ECTEOLA | pure anion exchange cellulose | Macherey, Nagel & Co. | Brinkmann Instr. |
| Cellulose Powder C-1000 | cellulose powder with plaster of Paris | Excorna O. H. G., Mainz, Germany | Brinkmann Instr. |
| Magnesium Silicate Woelm | pure magnesium silicate ¹ | Woelm | Alupharin |
| Polyamide MNP | pure polyamide powder | Macherey, Nagel & Co. | Brinkmann Instr. |
| Polyamide Woelm | pure polyamide powder ¹ | Woelm | Alupharin |
| “Hostalen S” | Polyethylene powder | Farwerke Hoechst, A.G., Frankfurt a.M., Germany | |
| “Florisil” | activated magnesium silicate | Floridin Co., P.O. Box 989, Tallahassee, Fla. | |
| “Sephadex” | cross-linked dextrans | Deutsch. Pharmacia, G.m.b.H., Frankfurt a.M., Germany | |
| Polyacrylonitrile | | Farbenfabriken Bayer, Dormagen, Germany | |
| “Perlon” | | Farwerken Hoechst, Bo- bingen, Germany | |
| ε-Polycaprolactam resin | | Toyo Rayon Co., Nakano- shima, Kita-ku, Osaka, Japan | |
| “Dowex” 1 and 50 | | Dow Chemical Co., Mid- land, Mich. | |
| “Celite” No. 545 | anion and cation exchangers diatomaceous earth | Johns-Manville, Interna- tional Corp., New York | |

¹ These adsorbents are of an especially fine particle size for thin-layer chromatography and produce mechanically stable layers without binders.

CHAPTER 3

Preparation of Thin Layers

INTRODUCTION

The thin layers of adsorbent used for thin-layer chromatography are supported and held in place by glass plates. The plates may, in theory, be any convenient size, but in fact the size is usually dictated by the apparatus used to prepare the layers. Layers are applied to the plates by spreading or spraying a slurry, by spreading a dry powder, or in the case of small plates, by dipping them in a slurry. The thin film of slurry is dried to produce the thin layer. The layer may then be treated in various ways before it is used.

THE VARIABLES IN LAYER PREPARATION

In general, the variables to be considered are the nature of the adsorbent (Chapter 2), the type of glass support plate, the thickness of the layer and the moisture content.

The Glass Plates

The glass plates used in thin-layer chromatography may be flat and smooth, flat and lightly ground^{180,254,328,334} or ridged as shown in the cross-section diagram in Figure 3.1. Flat, smooth plates have been almost universally used, but the ground surfaces are said to increase the adhesion of the layers. The ridged plates are used solely to simplify layer preparation. Thus, one simply pours the adsorbent-water slurry on a ridged plate, wipes off the excess with a spatula and dries the finished layer. The squared-ridge plates (Figure 3.1a) have been advocated by Gamp, Studer, Linde and Meyer,¹¹⁴ but the rounded ones (Figure 3.1b) are more readily available from normal glass shops,

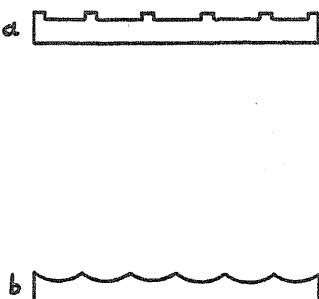


Figure 3.1. Cross-section views of two types of ridged glass plates.

where they are used to make semiopaque windows.* The ridged plates and the microchromatoplate system described later (p. 42) probably offer the most convenient systems for student use. To prevent accidents, all plates should have slightly beveled and smoothed edges.

The size and thickness of the glass plates are essentially dictated if a mechanical device such as those described below is chosen to prepare the layers. It has been found convenient to use, routinely, at least two sizes (exclusive of microplates). The smaller size should be wide enough to carry out concurrent, one-dimensional chromatography on three to four samples (about 2 in. or 5 cm minimum). Most workers allow the solvent front to rise 10 to 15 cm so that an over-all length of 20 cm or 8 in. is satisfactory. The larger size should be a square plate suitable for two-dimensional chromatography of a single sample or simultaneous, one-dimensional chromatography of eighteen or more samples. In a commercial apparatus system, the dimension of the square plate is equal to the length of the small plate (20 cm or 8 in.) and a multiple of its width. Such two-size systems are available with Stahl-type apparatus from Desaga-Brinkmann (p. 37) (5×20 cm and 20×20 cm) and Research Specialties Co. (p. 40) (2×8 in. and 8×8 in.). The Kirchner-type** apparatus manufactured by

* The author's attention was first called to the use of rounded plates by Dr. Louis Long of the Quartermaster Research and Development Laboratories in Natick, Mass.

** The author has elected to give this name to the type of apparatus in which the glass plate is passed under a stationary hopper because the Kirchner group was the first one to describe such an apparatus.^{25a}

Camag (p. 36) is normally designed for only one width, but two sizes of apparatus (10 cm and 20 cm width) are available. On the other hand, the Kirchner-type apparatus can be used with any type and thickness of glass, while the Stahl design works best with the plates supplied by the manufacturer. Although soft glass plates are routinely used, "Pyrex" plates are available from the manufacturers of the spreading apparatus.

If layers are prepared by spraying or by any of the simpler techniques discussed below, the plates can be of any size or type.

The original "chromatostrips" of Kirchner, Miller and Keller¹⁹³ were only wide enough for one sample (0.5 in.) although these workers also used wide plates. Such small strips are prepared in an apparatus designed by Miller and Kirchner²⁵³ and are still used³⁸⁸⁻³⁸⁹ by Stanley and his co-workers. However, they preclude a concentration study in which the sample is applied to a single plate in several concentrations, as well as a more precise comparison of two or three samples. The narrow plates are, however, more suitable for descending chromatography (p. 68).

The use of microscope slides for the preparation of "microchromatoplates" was described by Meinhard and Hall²⁴⁶ in the first paper advocating the use of bound layers. Lately, the use of such plates has again become fashionable^{152,437} and Peifer,²⁹⁶ in an exceptionally fine paper, has described a complete system based upon microscope and lantern slides (see p. 42).

A somewhat different type of layer support was worked out by Lie and Nyc,²¹⁹ who coated the inside of test tubes with thin layers of adsorbent. Rosi and Hamilton³³⁰ have prepared layers on both sides of glass plates so that they may be utilized more efficiently.

The Thickness of the Layer

Within a reasonably wide range (0.15 to 2.0 mm) the thickness of the layers is unimportant with respect to *Rf*'s and the degree of separation. The thickness itself should be prescribed by the type of information or results sought. For diagnostic or qualitative work, very thin layers are best because the spray reagent is much more sensitive when it does not have to "search" for tiny amounts of substance in a large amount of adsorbent. On the contrary, when the work is pre-

parative in nature, it is more efficient to use as thick a layer as possible so that the maximum amount of material can be separated in a single chromatogram.

The minimum thickness of about 0.15 mm was determined by Stahl, Schröter, Kraft and Renz³⁸¹ through a study of *Rf* vs. thickness. Only above this value were the *Rf*'s relatively constant (on silica gel layers). The maximum thickness is determined by the difficulty of drying thick layers without cracking and the degree to which there is unequal migration on the top and bottom of a layer. Honegger¹⁵⁸ investigated layers up to 5 mm thick and concluded that the best results were obtained between 1 and 3 mm. Ritter and Meyer,³²⁹ in a similar study, obtained better results with 1-mm layers than with 2-mm layers. The vast majority of diagnostic work has been done with layers 0.25 mm thick because the original Stahl-Desaga apparatus produced layers of this thickness. The Kirchner group¹⁹³ used 0.02 in. (0.51 mm).

Thin layers of unbound adsorbents which are prepared by spreading the dry powders on a plate are generally somewhat thicker, ranging from 0.5 mm²⁸⁰ to 1 mm.⁵²

The Moisture Content

The activity of adsorbents to be used for adsorption chromatography is greatly reduced by small amounts of water. Since most thin layers are prepared from aqueous slurries, this variable is controlled largely by the length of time and the temperature used to dry and activate the layers. This will be discussed in detail in a later section entitled "Drying of Layers" (see p. 46).

LAYER PREPARATION

Cleaning the Glass Plates

It is essential that the glass plates be as clean as possible and that they be particularly free of grease. Normally, washing the plates with detergent and wiping them with cotton soaked with hexane just before application of adsorbent will suffice. Sometimes, however, it is necessary to clean the plates with some type of cleaning solution. Peifer²⁹⁰ has recommended that his microchromatoplates be washed

successively with detergent solution, water and finally with 50 per cent methanol-water slightly acidified with hydrochloric acid.

The Preparation of Slurries

As previously stated, the majority of thin-layers are prepared by spreading a uniform film of a slurry of the adsorbent on glass plates and allowing it to dry. Normally, the liquid portion of such a slurry is water, which may contain acids, bases, buffers or complexing agents, and the solid portion is adsorbent and binder. The major problem associated with these slurries is the obtaining of a viscosity suitable for the manner of spreading. If the slurry is too thin, it runs through the spreader too rapidly and produces excessively thin layers. When it is too thick, it does not run through the spreader rapidly enough and may clump.

When plaster of Paris is used as a binder, this viscosity is controlled by two factors: the relative amounts of adsorbent and water, and the time elapsing between the addition of water to adsorbent and the actual spreading operation. The hydration of plaster of Paris ($\text{CaSO}_4 \cdot \frac{1}{2}\text{H}_2\text{O}$) to gypsum ($\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$) is so rapid that the slurry becomes unmanageable in two to three minutes. When buffers or other modifying reagents are added to the slurry, this time is appreciably shortened.²³² The actual mixing of adsorbent and water can be carried out by shaking them in a stoppered flask, by triturating them in a mortar or by stirring them in some other vessel. Table 3.1 shows the amounts of water suggested for various commercially available adsorbents. When possible, the type of spreading device is also given. Commercial sources of the adsorbents have been cited in the preceding chapter.

For the preparation of very thick layers, Honegger¹⁵⁸ suggests that these ratios be slightly modified by decreasing the amount of water. Furthermore, for layers from 3 to 5 mm thick, he added an additional 2 per cent of plaster of Paris and dried the layers under an infrared lamp. His data are given in Table 3.2.

The procedure for preparing a slurry containing starch as a binder is essentially different in that it involves a heating step. Kirchner, Miller and Keller¹⁰³ give the following procedure for such a preparation containing phosphors.

"The specified amounts of material [19 g of Merck reagent grade

TABLE 3.1. RECOMMENDED AMOUNTS OF WATER TO BE ADDED TO VARIOUS COMMERCIAL ADSORBENTS

| Adsorbent | Adsorbent-water, w/v, g/ml | Apparatus | Ref. |
|---------------------------------------|---|-----------|--------|
| Silica Gel G | 30/60 | Desaga | Manuf. |
| Silica Gel Woelm ¹ | 30/45 | — | Manuf. |
| Silica Gel D5 | 20/50 | Camag | Manuf. |
| Silica Gel, "Anisil" | 15/30 | Desaga | Manuf. |
| Aluminum Oxide G | 25/50 | Desaga | 232 |
| Aluminum Oxide D5 | 20/65 | Desaga | 232 |
| | 20/50 | Camag | Manuf. |
| Aluminum Oxide D5F | 20/50 | Camag | Manuf. |
| Alumina Woelm ^{1, 2} | 35/40 | — | Manuf. |
| Kieselguhr G | 25/50 | Desaga | 377 |
| Cellulose MN 300 G | 15/100 | Desaga | 319 |
| Magnesium Silicate Woelm ¹ | 15/45 | — | Manuf. |
| Polyamide Woelm ¹ | 5 g in 45 ml of chloroform-methanol (2:3) | — | Manuf. |

¹ These adsorbents contain no binder, but are of an especially small particle size.² Same proportions for acid, basic or neutral.TABLE 3.2. HONEGGER'S DATA FOR PREPARATION OF VERY THICK LAYERS¹ *

| Adsorbent | Thickness, mm | Adsorbent-H ₂ O, g/ml | Air-dry, min | Infrared dry- ing, min |
|------------------|------------------|-------------------------------------|--------------|---------------------------|
| Silica Gel G | 1-1.5 | 1:1.7 | 5 | — |
| | 2-3 | 1:1.6 | 30 | 30 |
| | 3-4 ² | 1:1.6 | 30 | 30 |
| | 4-5 ² | 1:1.57 | 30 | 30 |
| Aluminum Oxide G | 1-1.5 | 1:1 | 5 | 30 |
| | 4-5 | 1:0.9 | 10 | 30 |

¹ The data were obtained using the Camag apparatus.² Added 2% more plaster of Paris.* Reproduced from Honegger, *Helv. Chim. Acta*, 45, 1409 (1962) through the courtesy of the author and Verlag Helvetica Chimica Acta, Basel, Switzerland.

silicic acid passing 100 mesh sieve, 1 g of "Clinco 15" starch, 0.15 g of zinc silicate and 0.15 g of zinc cadmium sulfide] thoroughly blended while dry, were mixed with 36 ml of distilled water in a 250 ml beaker. The slurry was then heated on a water bath held at 85° with constant stirring until it thickened (1.75 minutes) and was then held at this temperature for 30 seconds longer with stirring. The beaker was removed from the bath and 2 to 7 ml of water were added immediately to form a thin paste." As stated on p. 20, a mixture of corn

starch and "Superior AA Tapioca Flour" is better than "Clinco" starch. If the amount of starch is decreased by half, it is not necessary to watch the temperature during the heating. The mixture must only be warmed thoroughly to thicken it.

Although water is the most common liquid for the preparation of slurries, several workers have preferred nonaqueous systems. In addition to the test-tube system of Lie and Nyc²¹⁹ and the micro-chromatoplate system of Peifer,²⁹⁶ which are quoted elsewhere in this chapter, Hörhammer, Wagner and Bittner¹⁶⁰ have used ethyl acetate, Duncan⁹⁰ has used water-methanol, 1:1, v/v, and Müller and Honerlagen²⁷⁰ have used acetone. None of these authors used the conventional spreading apparatus.

Mechanical Devices for the Preparation of Layers

At the present time, these devices fall into three major categories, the Kirchner type (see footnote on p. 31), the Stahl type and the spray type. While these "gadgets" are considerably more expensive and complicated than those used in some of the methods which will be described later, the author believes they have a distinct advantage. They produce uniform layers of any desired thickness in a very efficient manner.

The Kirchner Type. This type of spreading device is characterized by a stationary reservoir or hopper which deposits a thin film of adsorbent slurry on glass plates which are slowly passed under it. The layer-thickness is determined by the distance between the plate and the back edge of the hopper, and the width of the plates is predetermined by the size of the apparatus. The first such apparatus was designed by Miller and Kirchner in 1954²⁵³ to produce "chromatostrips" 0.5 in. wide. The apparatus has since been modified by Applewhite, Diamond and Goldblatt⁸ and shop drawings of the modification are available from the authors (Western Regional Research Laboratory, U. S. Department of Agriculture, Albany, Calif.).

A commercial model of this type of apparatus is shown in Figure 3.2 and is manufactured by Camag* in Switzerland. It is based upon the design of K. Mutter and J. F. Hofstetter at Hofmann-LaRoche A.

* Camag A. G., Homburger Str. 24, Muttenz, B. L., Switzerland. U. S. representatives: A. H. Thomas Company, Vine Street at 3rd, Philadelphia, Pa. and Microchemical Specialties Co., 1825 East Shore Highway, Berkeley 10, Cal.

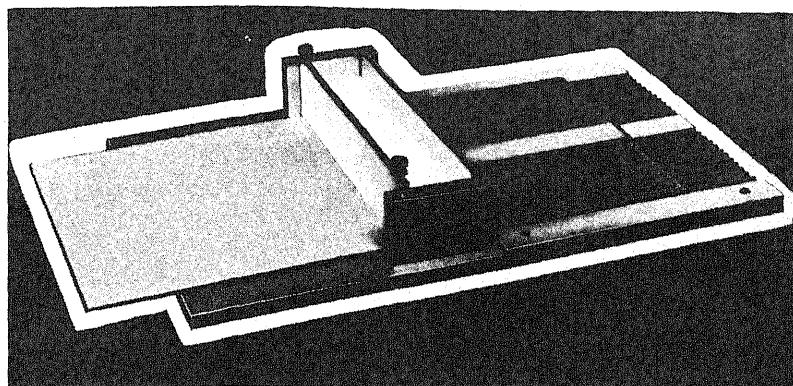


Figure 3.2. The commercial model of the Kirchner-type apparatus as designed by Mutter and Hofstetter. (Reproduced through the courtesy of Camag A. G.)

G. in Basel. Two models of this apparatus are available; one of these (Model A) coats plates 20 by 10 cm for one-dimensional chromatography and the second (Model B) coats plates either 10 cm or 20 cm in width. The Camag apparatus has been slightly modified by Wollish, Schmall and Hawrylshyn.⁴⁵² A similar apparatus is manufactured by the Kensington Scientific Corp.* in California.

It would appear to the author (who has had no experience with this apparatus) that the Kirchner type would be somewhat more tedious to use and less flexible with respect to plate width, than the Stahl type. On the other hand, it is less sensitive to plate thickness and probably yields more consistently uniform layers on small plates.

The Stahl Type. This type of spreading device is characterized by a moving slurry reservoir and stationary plates. The plates are arranged in a continuous surface on a support (the commercial models use plastic slabs) having a raised edge on two sides to hold the plates in position during the coating operation. The reservoir, machined in such a way that it produces the desired layer thickness, is then passed over the plates.

The first commercial model of this design was worked out by Stahl and is manufactured by Desaga** in Germany. The apparatus is pic-

* Kensington Scientific Corp., 1717 Fifth St., Berkeley, Cal.

** C. Desaga, G.m.b.H., Hauptstrasse 60, Heidelberg, Germany. U. S. representative: C. A. Brinkmann and Co., Inc., 115 Cutter Mill Rd., Great Neck, N.Y.

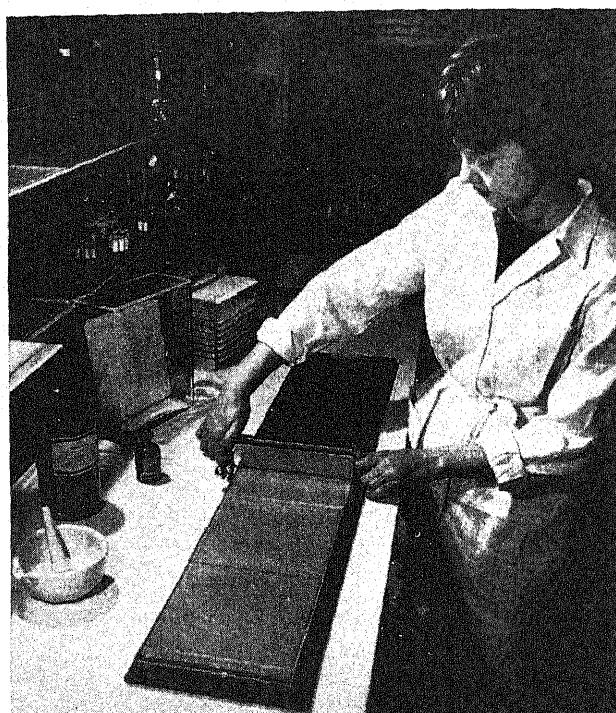


Figure 3.3. The Stahl-Desaga apparatus showing, in detail, the variable thickness applicator, a, and the standard applicator, b. (Reproduced through the courtesy of Brinkmann Instruments, Inc.)

tured in Figure 3.3. The spreader is available in two models; the Standard Model which gives layers 0.25 mm thick and the Model S 11 which gives layers of any thickness up to 2 mm. The standard plate sizes are 5×20 cm and 20×20 cm. Twenty of the small plates or five of the large ones can be coated in a single operation. While the technique works beautifully with the larger plates, the author has had difficulty obtaining uniform layers on small plates. This difficulty has also been encountered by Bennett and Heftmann²⁵ who solved it by placing a series of the large size plates (20×20 cm) under the small ones.* A glass slab or a smooth desk top can also alleviate

* Shandon Scientific Co., Ltd., 65 Pound Lane, London, N.W. 10 (U.S. representative, Consolidated Laboratories, Inc., P.O. Box 234, Chicago Heights, Ill.) manufactures an apparatus to avoid this difficulty. It contains a special leveling device and layer thicknesses are guaranteed to ± 0.010 mm.

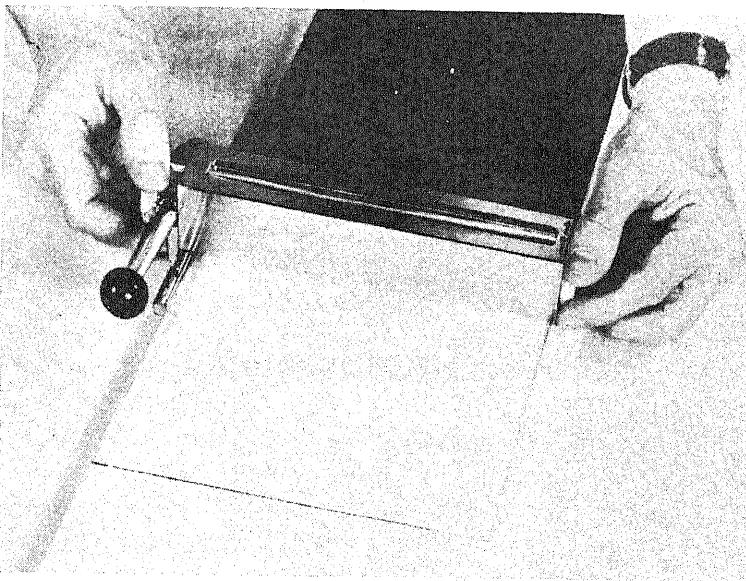


Figure 3.3a.

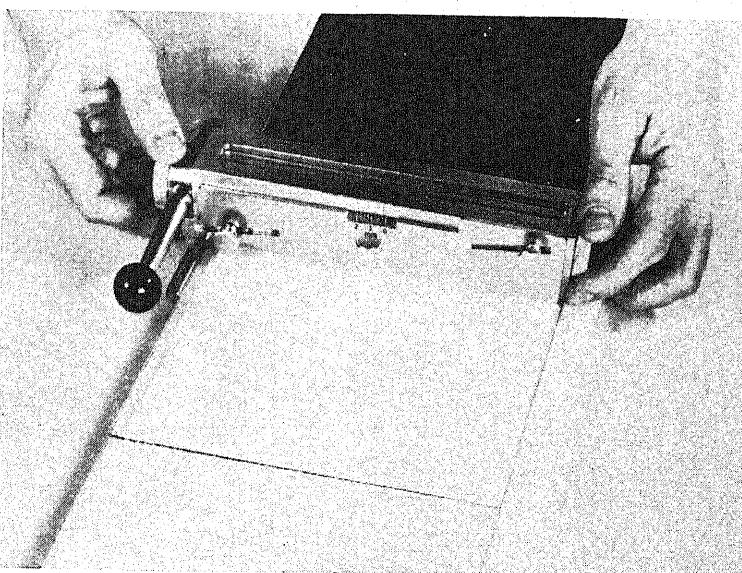


Figure 3.3b.

the problem. Sometimes wavy layers are produced by the "bump" caused by going from one plate to the next. As long as the developing solvent is passed in a direction parallel with the spreading operation, this is no problem.

A second commercial model similar to the Desaga apparatus is manufactured in the United States by Research Specialities Co.* This apparatus can also be purchased in a standard model or a variable thickness model. It is shown in Figure 3.4.

Home-made apparatus of the Stahl type has been devised by Barbier, Jäger, Tobias and Wyss,¹³ Machata,^{227**} Gamp, Studer, Linde and Meyer¹¹⁴ and Schulze and Wenzel.³⁴⁹

Sprayed Layers. The notion of spraying a thin slurry of adsorbent on glass plates has been suggested, notably by Reitsema,³²³ who used a small paint sprayer. Bekersky²⁴ has worked out a procedure using a normal laboratory spray bottle. Dilute slurries of Silica Gel G (15 g to 35 ml of water) or Aluminum Oxide G (20 g to 50 ml of water) are sprayed from a distance of 7 to 9 in. onto plates in a horizontal position. The method produces fairly uniform smooth layers, but the final thickness is unknown and only approximately controllable. The method has not yet been commercialized.

"Poor Man's Layers"

The development of a cheap method for the preparation of uniform, thin layers has challenged the ingenuity of chemists everywhere. This has resulted in the evolution of several acceptable methods. While the author is a firm believer in the more complex spreaders, these methods certainly offer possibilities for student use and for persons who wish to explore the system before investing in an apparatus.

The simplest way of preparing a layer of slurry is surely that of Lees and DeMuria²¹⁴ as shown in Figure 3.5. One to three layers of tape, either surgical or masking, but not "Scotch," are put on two opposite edges of a single glass plate or on the outside edges of the outside plates of a set arranged on a flat surface. The slurry is poured

* Research Specialty Co., 200 South Gerrard Blvd., Richmond, Calif.

** A plastic apparatus of this type is manufactured by Applied Science Laboratories, College Park, Penn.

on an open end and smoothed by drawing a glass rod over the plate or plates in a direction parallel with the taped edges. The tape supports the rod and determines the layer thickness. After a few minutes, the tape is removed and the layers are dried. The method is not very satisfactory for preparing thick (ca. 1–2 mm) layers. A similar method was devised by Duncan⁹⁰ who used removable metal strips on the edge of the plates to support the leveling rod. In this case, the thickness of the metal determines the thickness of the layer. Duncan used methanol-water, 1:1, v/v, to prepare his slurries. The detail of such a strip is shown in Figure 3.6. Boll⁴¹ arranged a set of plates in an apparatus with raised edges. The slurry is poured on and leveled with a straight edge. Still another method is to machine out an indentation in a large spatula to a depth corresponding to the desired thickness. Such a spatula (Figure 3.7) is then used to level a slurry on a glass plate which is large enough to support the two ends of the blade.* As in many other aspects of thin-layer chromatography, the first mention of this type of thing was made by Kirchner, Miller and Keller¹⁹³ who used an arrangement of glass plates, some placed 0.02 in. above the others and serving as support for a leveling rod. Thus, this concept is as old as the method.

Still other workers prefer to pour a slurry of adsorbent in ethyl acetate on a plate and to level it by tipping it.¹⁶⁰ Such layers are said to require no activation and to function satisfactorily in a chamber which is not saturated. The same procedure has been used with aqueous starch-silica gel slurries.⁴³ A similar scheme was used by Wang to prepare polyamide layers.^{436a} He prepared 20 per cent solutions of polyamide in 80 per cent formic acid, poured these solutions on glass plates, leveled them by tipping and allowed them to dry.

A special problem arises with the preparation of thin layers on the inside walls of test tubes.²¹⁹ Such layers were prepared and used by the following method. The test tube to be coated (13.5 × 150 mm) is filled with a suspension of silicic acid (no binder) and chloroform (1:2.5, w/v) and allowed to stand for about 5 seconds. The contents are then slowly poured out of the tube in one continuous slow tilting motion. After most of the slurry is discharged, the vessel is held in an

* This method was mentioned to the author by Mr. Robert Conkin of the Monsanto Chemical Co., St. Louis, Mo.

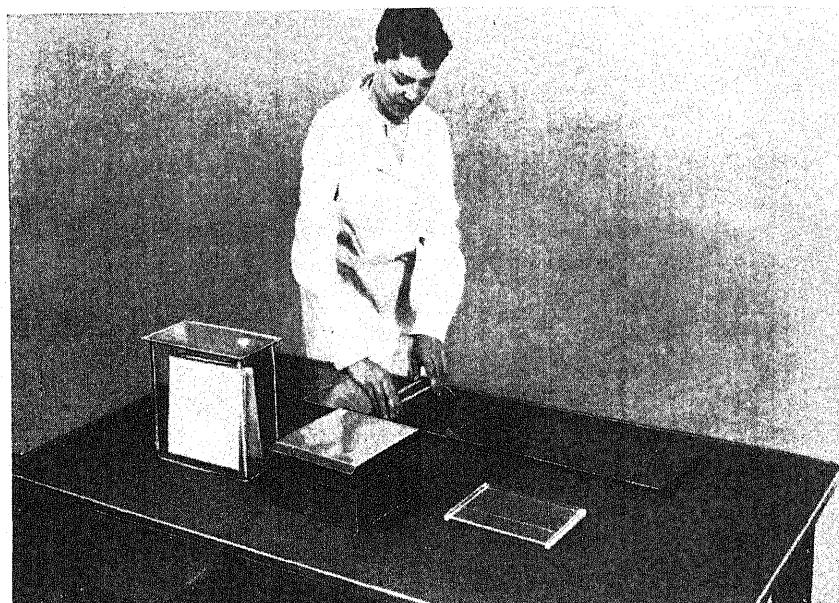


Figure 3.4. The Research Specialities apparatus showing, in detail, the variable thickness applicator, a, and the standard applicator, b. (Reproduced through the courtesy of Research Specialities Co.)

inverted position for 2 to 3 seconds and then slowly tilted, without rotation, to a horizontal position for drying. The top portion (in the horizontal position) is marked because it is the linear surface where the sample will be placed. An additional coating is placed on the open end of the test tube by immersing the tip end about 5 mm, at an angle of 45°, in the adsorbent slurry. In this position it is rotated once. The sample is placed 1 cm from the open end on the side previously marked. The open end is dipped in a suitable developing solution.

Microchromatoplates

Thin layers have been prepared on microscope slides and lantern slides by spreading a paste with a spatula²⁴⁶ with an especially designed gadget (for microscope slides only)⁴³⁷ and using the Stahl apparatus with an alternate arrangement to hold the slides during the slurry application.¹⁵² The author has prepared satisfactory layers using the complete Stahl assembly.

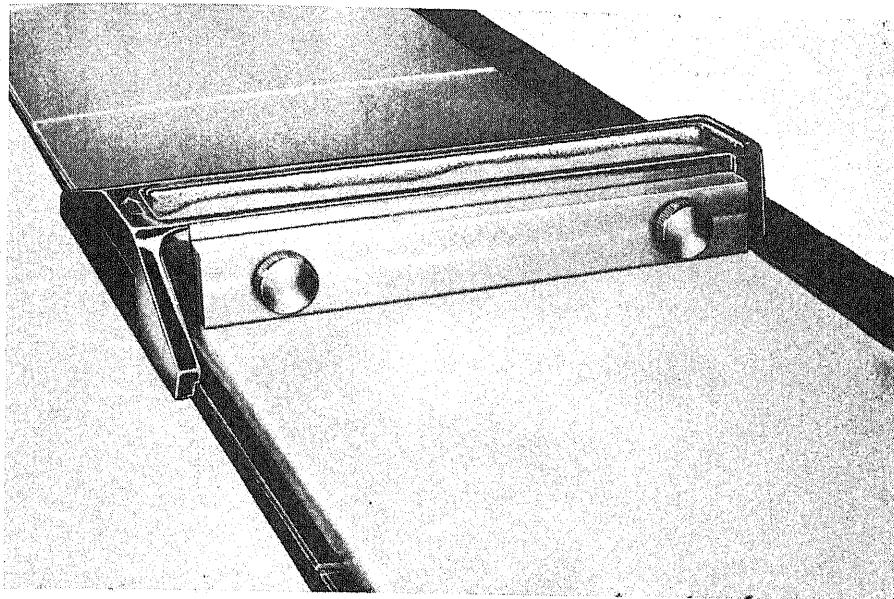


Figure 3.4a.

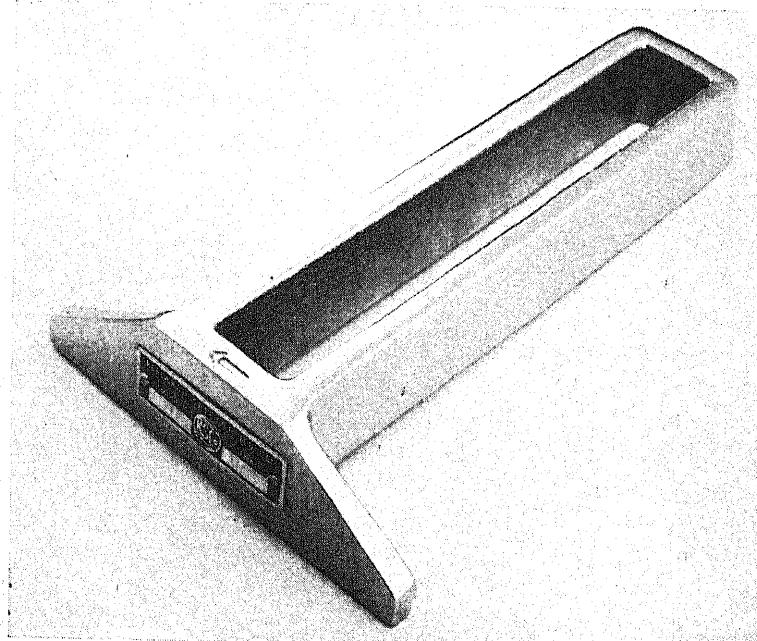


Figure 3.4b.

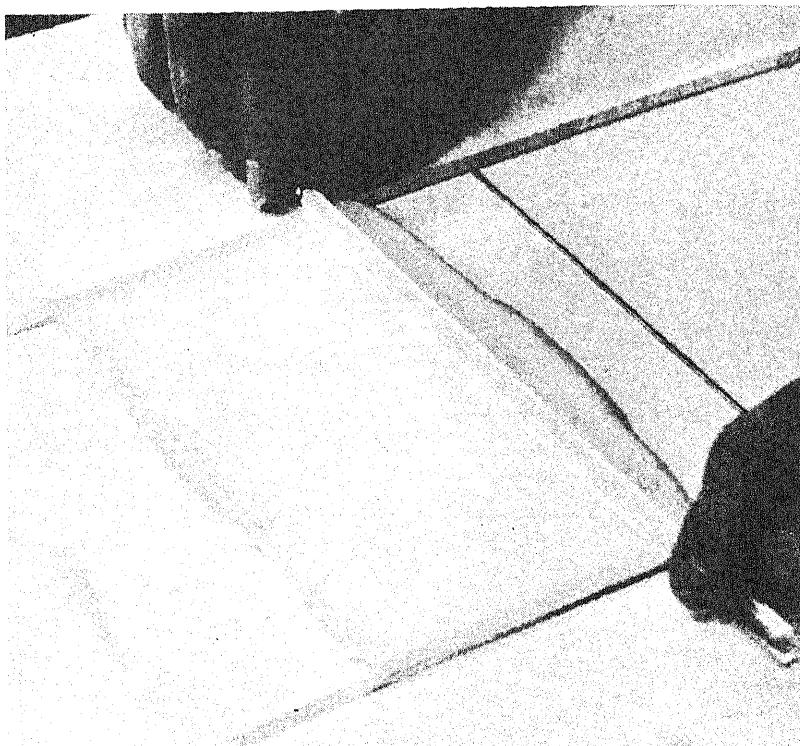
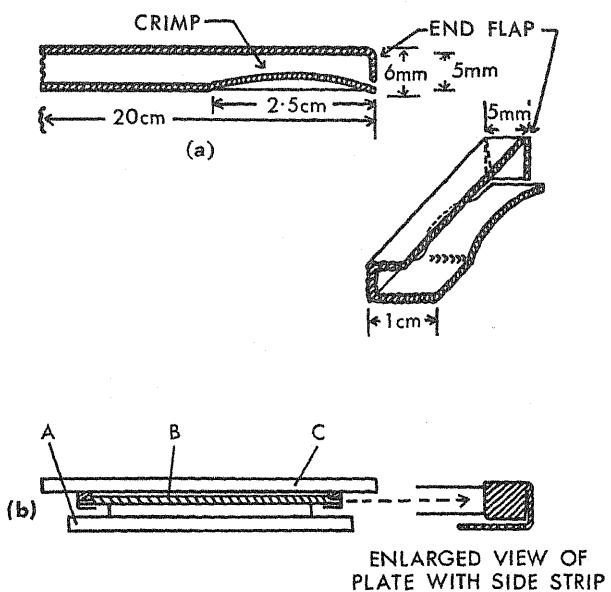


Figure 3.5. Preparation of thin layers using tape and a glass rod. (Reproduced from Lees and DeMuria, *J. Chromatog.*, 8, 108 (1962) through the courtesy of the authors and the Elsevier Publishing Co.)

Peifer²⁹⁶ has developed a complete scheme for chromatography on such small plates. He uses microscope slides for one-dimensional work and lantern slides for two-dimensional work. The slides, cleaned with detergent, water and water-methanol (50:50) acidified with hydrochloric acid, are *dipped*, two at a time, back to back, into a slurry of adsorbent in a nonaqueous medium. The slurry should be well stirred (about 1 min.) before dipping. They are then lifted slowly out of the slurry and allowed to drain on the edge of the container. They dry rapidly, are separated and the edges are wiped clean with tissue. In order to obtain a more durable layer, the plates are exposed briefly to steam to hydrate the plaster of Paris binder. The steam treatment is not actually necessary with silica gel layers. The layers are then



ENLARGED VIEW OF PLATE WITH SIDE STRIP

Figure 3.6. Detail of Duncan side strips, a, and the side strips in use, b. The side strips are fastened to the opposite edges of a glass plate (B) and held in place by the 2.5 cm long indentation. Slurry is poured on the plate and leveled with a glass rod (C) which rests on the side strips. The apparatus is shown on a mounting board (A). The thickness of the metal in the strips determines the layer thickness. (Reproduced from Duncan, *J. Chromatog.*, 8, 37 (1962), through the courtesy of the author and the Elsevier Publishing Co.)



Figure 3.7. A machined spatula for the preparation of thin layers.

activated by suspending them about 6 mm over a hot plate for 1 to 3 min. As mentioned previously, Peifer sometimes added sulfuric acid to his adsorbents to facilitate visualization. The recipes for Peifer slurries are summarized in Table 3.3. The chromatograms containing sulfuric acid are visualized by warming them over a hot plate and the others are sprayed in the normal manner.

TABLE 3.3. RECIPES FOR PEIFER SLURRIES²⁶⁵

| Adsorbent | Slurry Medium | Proportions, g in ml |
|--|--|-----------------------------|
| 1. Silica Gel G | chloroform or chloroform-methanol (2:1, v/v) | 35 g in 100 ml |
| 2. Silica Gel G and sulfuric acid | chloroform-methanol-sulfuric acid (70:30:2.5, v/v/v) | 50 g in 102.5 ml |
| 3. Cellulose powder ¹ | chloroform-methanol (50:50, v/v) | 50 g in 100 ml ¹ |
| 4. Alumina ² | chloroform-methanol (70:30, v/v) | 60 g in 100 ml ² |
| 5. "Florisil" ³ (60-100 mesh) | chloroform-methanol-acetic acid (70:30:1, v/v/v) | 55 g in 101 ml ³ |

¹ 35 g of cellulose powder and 15 g of plaster of Paris are triturated with a minimum volume of methanol. The resulting viscous mixture is then diluted to give the above ratio.

² 45 g of activated alumina and 15 g of plaster of Paris are triturated with a minimum volume of chloroform-methanol and then diluted to the above ratio.

³ 45 g of "Florisil" and 10 g of plaster of Paris are triturated with 1 ml of glacial acetic acid and a minimum volume of chloroform. The result is diluted to the above ratio.

Layers Prepared From Dry Powders

The preparation of layers from dry powders of unbound adsorbents is a slightly different problem. The first method was that of Mottier and Potterat^{267,268} who used the apparatus shown in Figure 3.8. An even simpler affair is illustrated in Figure 3.9. A glass rod equipped with rubber or tape rings to hold it slightly above the plate and a larger ring to keep it centered is rolled over the plate containing an excess of alumina. This latter system is, of course, based upon the Mottier work, but has been more widely used.^{52,71,74,144,146}

Drying of Layers

After the slurry is spread on a plate, it should be allowed to stand for at least 30 min. so that the binder can "set." Indeed it is even recommended by several workers^{46,47,249,319} that more durable layers will result if they are allowed to stand in the air overnight. This would prevent cracking due to "casehardening." For adsorption chromatography, the layers are activated by heating them at 100 to 110° for various periods of time, generally one to several hours. There is little change in the activity when the time is lengthened beyond one hour or the temperature is raised above 110°. For layers containing plaster of Paris, the maximum drying temperature is 128° which is the point

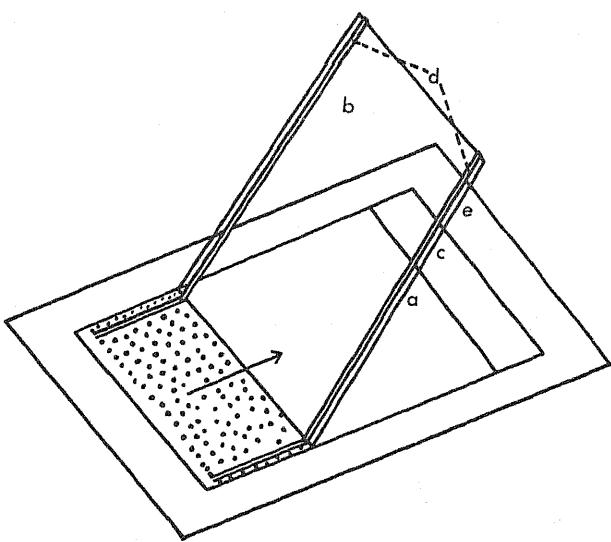


Figure 3.8. Preparation of thin layers of dry, adsorbent powders by the method of Mottier and Potterat. The dry alumina is placed on the glass plate a in front of the moving and leveling guide b. Plate c is a stopping board and d is two rubber bands which determine the layer thickness. Guide b is then moved over the plate. (Reproduced from Mottier, *Mitt. Gebiete Lebensm. Hyg.*, 49, 454 (1958) through the courtesy of the author and the Eidgenoessische Drucksachen-und Materialzentrale, Bern, Switzerland).

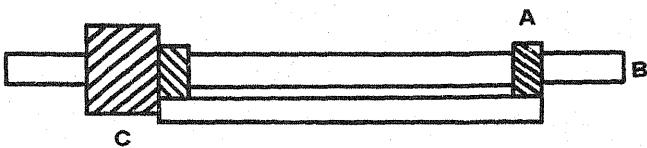


Figure 3.9. End view of a glass-rod leveling device for preparing layers of dry powders. The small spacers (A) are rubber tubing rings placed on a glass rod (B). The large ring (C) centers the gadget. Dry powder is placed on a plate and leveled by rolling the device back and forth.

at which gypsum again dehydrates. For partition chromatography, the layers are normally not activated since the residual water acts as the stationary liquid phase.

Better results with less cracking will be obtained if the layers are

dried in a vertical position as suggested by Desaga. All of the manufacturers of thin-layer equipment sell a metal drying rack which will hold several plates and which can be placed in the drying oven in either a horizontal or vertical position.

The activated plates should be stored in a desiccator or dry box of some type until they are used. The manufacturers of thin-layer equipment provide such containers. However, in the author's laboratory, the plates are carefully stacked in a normal desiccator over calcium chloride or, if large, are kept in a large box containing a beaker of sulfuric acid. After removal from the desiccator, the layers remain active for a time which is inversely proportional to the relative humidity. Normally, plates are active for ten minutes or more, but it is not wise to leave a plate out for several hours during the monitoring of a reaction or the elution of a column.

Other Post-Preparative Operations

The adsorbent sticking to the edges of the plates should be carefully wiped off before using the layers. The layers may then be pre-washed^{388,389} with an appropriate solvent. A 0.5 per cent solution of "Versene" (ethylenediaminetetraacetic acid trisodium salt) was used by Schweiger³⁵⁰ to prewash unbound cellulose layers. Figure 3.10 shows a suggested³⁸⁹ apparatus for carrying out this operation.

The finished plates may, at this stage, be sprayed with a complexing agent such as silver nitrate or boric acid.²⁶⁰ This serves the same purpose as the incorporation of these substances in the slurry as discussed in Chapter 2, but spraying is more convenient in that the plates can be prepared one at a time as needed or in that only a portion of a plate need be treated. Morris²⁶⁰ sprayed with a saturated aqueous solution of silver nitrate or a saturated methanolic solution of boric acid or both. After spraying, the plates were dried at 110° for one hour.

On occasion, it is desirable to use partition chromatography in which the polar, stationary liquid is something other than water. To this end, Teichert, Mutschler and Rochelmeyer^{399,400} have impregnated cellulose layers by dipping them into a 20 per cent (v/v) solution of formamide in acetone. Vaedtke and Gajewska sprayed layers of plaster of Paris bound "Celite" with formamide, propylene glycol

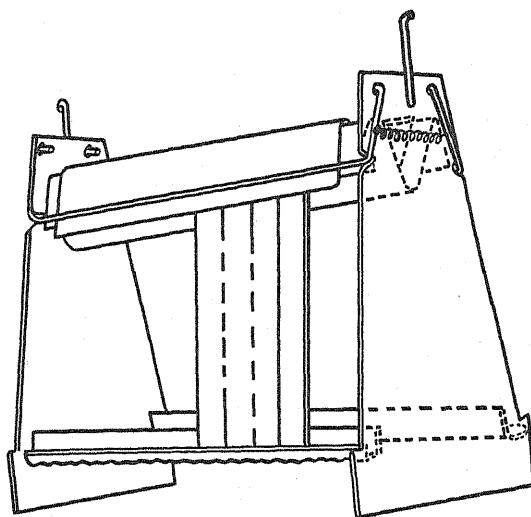


Figure 3.10. An apparatus for prewashing layers. The layers are placed in the apparatus and the washing liquid flows out of a reservoir by means of filter paper and over the layers in a descending manner. (Reproduced from Stanley, Vannier and Gentili, *J. Assoc. Off. Agr. Chemists*, **40**, 282 (1957) through the courtesy of the authors and the Association of Official Agricultural Chemists.)

or paraffin oil.⁴¹⁴ The plates were weighed before and after the spraying to determine the extent of impregnation. Knappe and Peteri²⁰⁰ added polyethylene glycol (15 g to 30 g of Kieselguhr G and 45 ml of water) to the adsorbent slurry before the layers were prepared. In the latter case 0.050 g of sodium diethyldithiocarbamate was also added to prevent peroxide formation in the polyglycol. Such chromatograms are developed with nonpolar solvent systems.

The Preparation of Reversed Phase Chromatograms

The impregnation of thin layers with a high-boiling, nonpolar liquid such as Silicone fluid or a hydrocarbon, leads to a partition chromatogram in which the moving phase is more polar than the stationary phase. Such chromatography is called reversed phase chromatography and normally gives improved separations in a homolo-

gous series of nonpolar compounds. This has been particularly adapted to the fatty acid series.

Malins and Mangold^{230,232} prepared Silicone layers by slowly immersing Silica Gel G layers in a 5 per cent solution of Silicone Oil* in ethyl ether. The operation is carried out at room temperature and both the plates and the liquid must be at this same temperature. In addition, the operation must be carried out slowly. Failure to observe these precautions will produce crumbly layers.

Kaufmann, Makus and Khoë,^{182,186} and Kaufmann and Ko¹⁸¹ have used either a 10 to 15 per cent solution of undecane in petroleum ether (b.p. 40 to 60°) or a 5 per cent solution of tetradecane in the same solvent. Reversed phase layers have been made using paraffin by Winterstein, Studer and Rüegg⁴⁴⁹ (5 per cent in petroleum ether) and by Michalec, Šulc and Měšťan²⁴⁹ (0.5 per cent in ether). All of these were prepared by dipping.

Purdy and Truter³¹⁵ prepared such layers by allowing Silica Gel G layers to be "developed" with a 15 per cent solution of decane in petroleum ether. A similar process was carried out by Wagner, Hörhammer and Dengler using 5 per cent paraffin in ethyl ether.⁴³¹

After the impregnation, the layers are allowed to dry in the air for a few minutes. The development is carried out with polar solvents.

* Dow-Corning 200 fluid viscosity 10 cs. Dow-Corning Corp., Midland, Mich. This preparation is also available from Research Specialities Co. (p. 40) in a spray package for spraying layers.

CHAPTER 4

Application of the Sample

INTRODUCTION

The various factors to be considered in the application of samples to thin layers are the application solvent, the amounts to be applied and the mechanics of the application. These are largely controlled by the type of information or results desired. Thus, for diagnostic or qualitative chromatography a small amount of substance, which need not be accurately known, is applied to a single small spot. This is then developed simultaneously with spots containing possible components of the mixture (reactants, products or known constituents). For quantitative chromatography, a precise amount of the mixture to be analyzed must be applied. For preparative chromatography, large quantities of the mixture to be separated must be applied in a thin uniform band along one edge of a plate.

After the sample has been applied, it can be caused to undergo various reactions which will assist in its identification or will reveal something of its properties.

VARIABLES IN SAMPLE APPLICATION

Application Solvent

The solvent used for the application of samples to thin layers is not particularly critical, but the following factors should be considered. The ideal solvent should boil between 50° and 100° so that it does not evaporate too rapidly but can be easily removed at will. It should completely dissolve the sample to yield at least a one per cent solution, but should be as nonpolar as possible. This allows the

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sample to be concentrated at the center of the spot and not in a ring around the edge. The application solvents should, in any case, be removed as completely as possible before the chromatogram is developed.

Amount of Sample Applied

The easiest and best way to ascertain this quantity is by experimentation with the specific compounds in question. Every compound or mixture should be investigated at several different concentrations. This can be done on a single plate and, simultaneously, information about trace impurities, relative concentrations and separabilities can be obtained. An amount is then chosen which is large enough to be effectively visualized and to show trace impurities, and small enough to give discrete spots with a minimum of tailing. This amount will depend upon the layer thickness and the sensitivity of the visualization procedure which are inversely proportional to each other. Further, it will depend upon the adsorbent and whether the chromatography is adsorption or partition. The former has a much higher capacity. When the sample is too large, tailing³⁰⁷ and high *Rf* values³⁵² will result.

For diagnostic and quantitative chromatography, samples ranging from 10 to 100 γ are normally used with layers about 0.25 mm thick. For preparative work, amounts ranging from 10 to 300 mg can be separated on a single chromatogram depending upon the size of the plate, the thickness of the layers, the ease of separation, and the type of chromatography.

Specific examples of quantitative and preparative thin-layer chromatography will be given in the chapters devoted to these subjects.

MECHANICS OF APPLICATION

The application of samples to bound layers is carried out by touching the tip of a filled capillary, micropipette, or microburette to the adsorbent layer in a manner analogous to that used in paper chromatography. Multiple applications with solvent evaporation after each are usually necessary. The sample is placed or "spotted" about 2 cm or 1 in. from the end of the plate so that the solvent level will be at least 1 cm below the center of the spot. The diameter of the

spot should not exceed 0.5 cm and should be as small as possible. The spot can be kept small by a rapid evaporation of solvent using a stream of dry nitrogen²³² or a low-temperature hot plate.^{223,244,253,329} Honegger¹⁵⁷ and Wagner, Hörhammer and Wolff¹³² advocate the application of the sample in thin bands about 1 cm long rather than the conventional circular spots. The bands are at a 90° angle to the direction of development. Tate and Bishop³⁹⁸ applied samples with a thin wire loop (about 1 mm in diameter) rather than the usual capillary and Metz²⁴⁷ used a paper gadget to make the application point very small.

The manufacturers furnish a "spotting template" to help with the application of a large number of samples. This is a transparent sheet of plastic which fits over a thin-layer plate but does not touch the layer. It is suspended on two side pieces and is marked at 1-cm intervals. The Desaga-Brinkmann model is shown in Figure 4.1.

When a large number of samples must be applied over a long period of time, it is a good idea to cover the layer above the sites of application with a clean glass plate to protect it from moisture.⁴⁶

Diagnostic Chromatography

For diagnostic work, the sample is usually applied through a melting point capillary tube of indeterminate size or a micropipette calibrated in microliters. Such a pipette is furnished by all manufacturers of thin-layer equipment.

Quantitative Chromatography

For this purpose, precise application methods are necessary. A microsyringe* used as such or with a mechanical push-button dispenser (Brinkmann, p. 37) is shown in Figure 4.2. The Agla** micrometer syringe has also been widely used. An appropriate microburette could be used.

Preparative Chromatography

The application of samples for preparative thin-layer chromatography is quite a different problem. Large quantities (50 to 300 mg)

* The Hamilton Company, Inc., Whittier, Calif.

** Burroughs Wellcome Co., The Wellcome Bldg., Euston Rd., London N.W. 1, England.

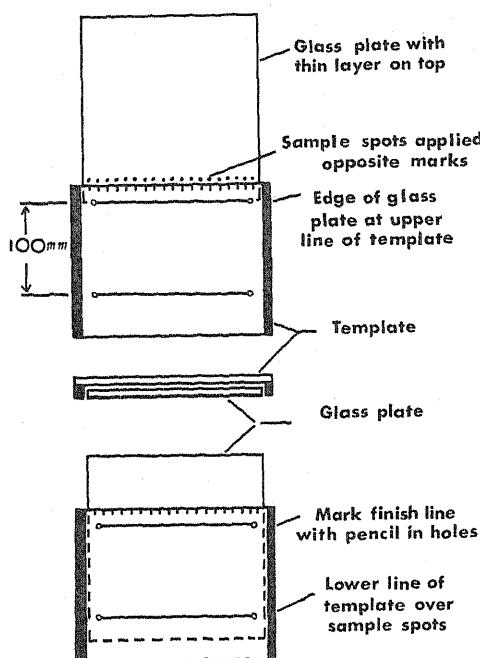


Figure 4.1. The Desaga-Brinkmann "spotting template" in position (Reproduced through the courtesy of Brinkmann Instruments, Inc.)

must be applied in a reasonable time as a uniform band without destroying the layer. Multiple spotting with a single capillary is quite unsatisfactory because it is so tedious and almost always results in a disturbed layer.

The simplest approach to this problem is to blow a fine jet of sample and solvent into the layer from a suitable pipette while moving the pipette in a uniform motion over the plate. The pipette should have a small orifice and the operator needs a deft pair of hands.* Honegger¹⁵⁸ has suggested that the sample be applied in a shallow V-shaped ditch made on the starting line. Care must be taken so that the ditch does not go through to the glass plate.

In addition to these methods, two mechanical gadgets are commer-

* The author has seen this done by Mr. P. J. DeMuria at the Chas. Pfizer Laboratory in Groton, Conn. and it has been reported elsewhere.

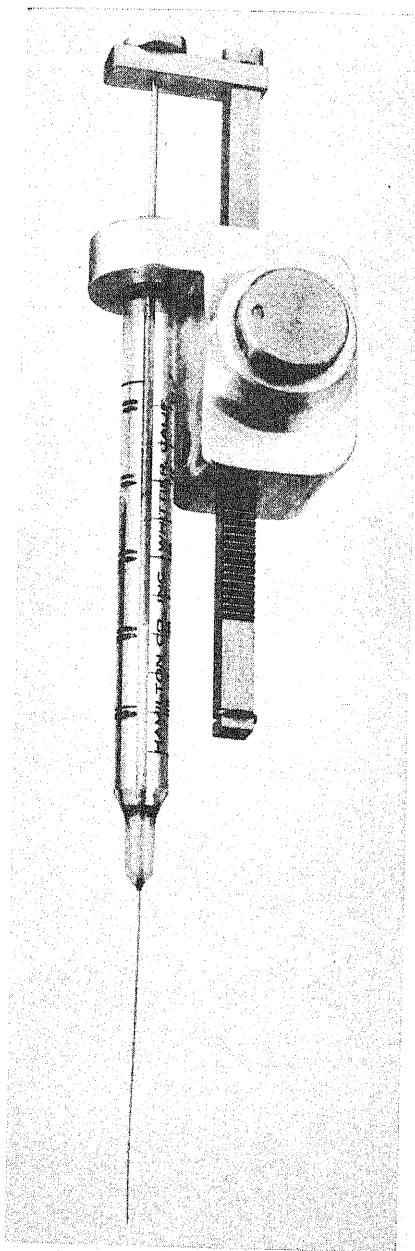


Figure 4.2. Microsyringe with push button dispenser for use in quantitative thin-layer chromatography. (Reproduced through the courtesy of Brinkmann Instruments, Inc.)

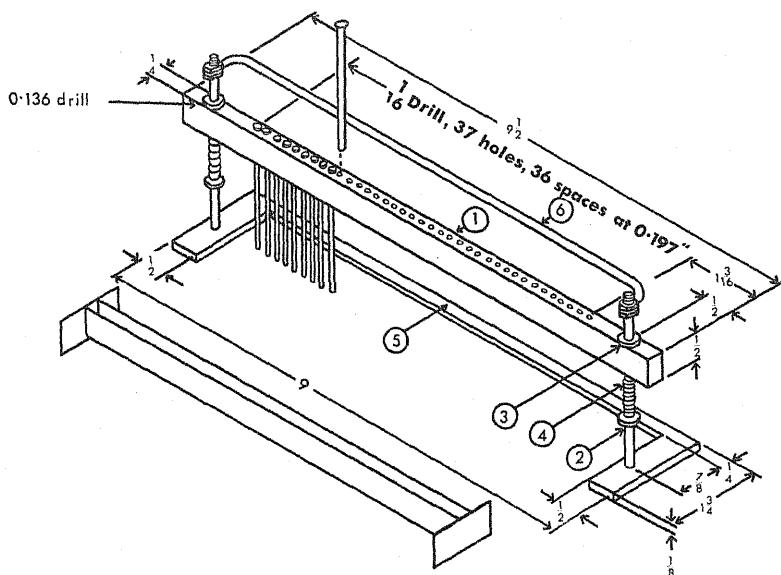
Thin-Layer Chromatography

Figure 4.3. The Morgan apparatus for sample application in preparative thin-layer chromatography. The apparatus consists of an aluminum bar (1) with holes drilled near its ends which permit it to slide freely on two vertical $\frac{1}{8}$ inch brass rods (2) and is held up against adjustable stop washers (3) by partially compressed springs (4). The top and bottom stop washers are $\frac{1}{4}$ inch OD and $\frac{3}{32}$ inch ID and are enlarged slightly with a punch so that they may be forced onto the vertical rods. The bottom washers which support the springs are soldered in place while the top washers remain in place by friction and may be adjusted by forcing either up or down. The two vertical rods threaded, screwed into tapped holes, and soldered in place in the cutout base plate (5) and the upper horizontal tie rod (6) form the rigid support for the apparatus. Thirty-seven precisely drilled $\frac{1}{16}$ inch holes spaced 0.197 inch (0.5 cm) on centers in the aluminum crossbar carry the spotting pipettes. These are made from pieces of 1.5 mm OD Pyrex melting point capillary tubing (Corning #9530) selected so that they will just pass through the holes in the crossbar with no friction. A funnel stop is blown at the upper end of each tube, the tubes cut at a length such that the tips will extend to within $\frac{1}{16}$ inch of the surface of the chromatographic plate when placed in the crossbar. The delivery tips are fire polished and squared off by grinding on a flat piece of fine carborundum wetted with water. Progress in the latter operation should be observed under suitable magnification and the grinding continued only until the contact surface of the tip is smooth.

A sample trough of suitable volume and design to be used with the apparatus in spotting preparatory plates may be constructed from metal, glass, or plastic depending upon the chemical nature of the samples and solvents employed. A trough of the design shown was constructed of No. 23 gauge Monel metal and has proven to be satisfactory for use with non-corrosive solvents. (Reproduced from Morgan, *J. Chromatog.*, 9, 379 (1962) through the courtesy of the author and the Elsevier Publishing Co.)

cially available for sample application in preparative chromatography. These are the multiple capillary arrangement of Morgan²⁵⁸ and the moving syringe system of Ritter and Meyer.³²⁹

The Morgan Apparatus. The Morgan apparatus is, simply, some thirty-seven capillary tubes arranged in a movable holder and is shown in Figure 4.3 with instructions for its construction. All of the tubes are filled by dipping them into a trough containing the sample in solution and they spot simultaneously when touched to a thin layer. It has been used with great success by the author and elsewhere at the University of Connecticut, and is commercially available from the A. H. Thomas Co.*

The Ritter and Meyer Apparatus. The Ritter and Meyer apparatus is a syringe equipped with a pressure valve that is supported by two bars on which it slides freely. The sample is placed in the syringe, a slight pressure is applied and the syringe is moved back and forth spraying a thin jet of sample. The plate holding the layer is placed on an asbestos-covered aluminum block which is heated to facilitate the removal of solvent. The apparatus is shown in Figure 4.4. It is commercially available from Desaga-Brinkmann (see p. 37).

Sample Application to Unbound Layers

The unbound layers (excluding the Woelm and Anisil special adsorbents) are not mechanically stable enough to touch so that samples are applied in small drops from a short distance away.⁵² When unbound layers are developed in a near horizontal position, the sample can be adsorbed on a small amount of adsorbent and placed on the thin layer as such.²⁶⁰ This latter technique is especially useful if one wishes to rechromatograph a sample on a new layer. The adsorbent containing the sample can simply be scraped off of one plate and applied to another without elution. Samples are applied to Woelm and Anisil layers in the same manner as with bound adsorbents.

CHEMICAL REACTIONS ON APPLIED SAMPLES

Sometimes, it is advantageous to modify or prepare a derivative of a sample after it has been spotted on a thin layer. This can be accom-

* Arthur H. Thomas Co., Vine Street at 3rd, Philadelphia, Pa.

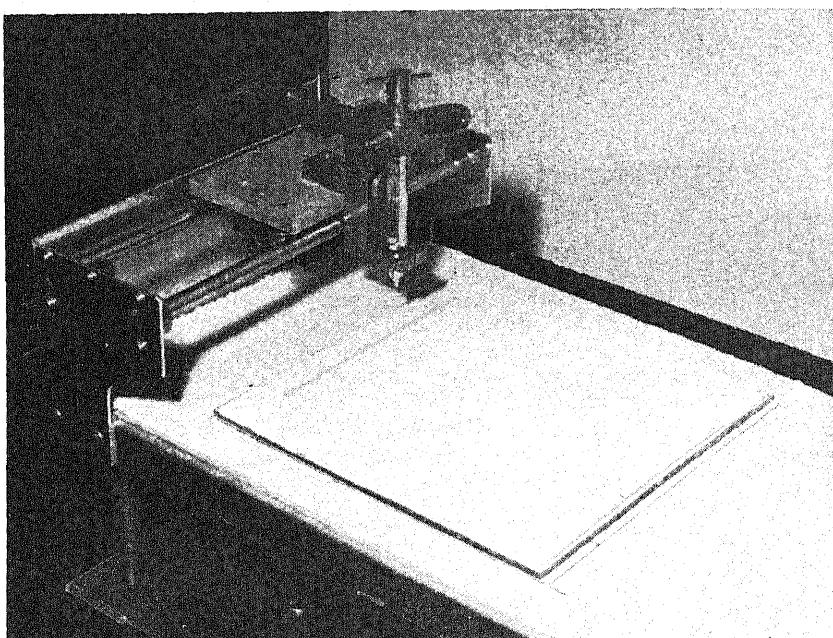


Figure 4.4. The Ritter and Meyer apparatus for sample application in preparative thin-layer chromatography. (Reproduced from Ritter and Meyer, *Nature*, 193, 941 (1962) through the courtesy of the authors and Macmillan and Co., Ltd.)

plished by adding the reagent to the developing solvent. Thus, bases can be regenerated from their salts and compounds can be brominated or oxidized by putting the appropriate reagents in the developing system. Alternately, a drop of reagent can be added to the spotted sample before chromatography. This technique has been used for oxidation, dehydration, hydrogenation and the preparation of derivatives. These reactions are carried out in the following manner.

Regeneration of Bases

The amine or alkaloid is spotted on the layer as its hydrochloride, and a base, diethylamine⁴³⁶ or ammonia,⁴³² is added to the developing solution to the extent of 1 or 2 per cent. A similar reaction was performed in the thin-layer separation of the halide ions⁴⁵⁷ by Seiler and Kaffenberger. The halides were spotted as their sodium and po-

tassium salts, but the developing solution contained 10 per cent of concentrated ammonium hydroxide and the ions migrated as their ammonium salts. Mottier²⁶⁶ neutralized dye solutions before chromatography on alumina by placing a drop of 1 N sodium hydroxide solution on the layer, drying it and then spotting the sample.

Bromination¹⁸⁶

The sample is spotted in the normal way and the chromatogram is developed by solvents containing 0.5 per cent (by volume) of bromine.

Oxidation

Oxidation is brought about by addition of an oxidizing agent to the developing system or by direct application over the sample spot. Thus, Mangold^{230,231,232} separated saturated and unsaturated fatty acids on reversed phase layers with a mixture of acetic acid-peracetic acid-water (15/2/3). The unsaturated compounds were oxidized and moved to the solvent front whereas the saturated compounds were separated in a normal manner.

In an alternate technique,²⁵² the sample to be oxidized is spotted in the normal way and covered with a drop of glacial acetic acid, saturated with chromic anhydride. The chromatogram is then developed with solvents which are not easily oxidized.

Dehydration²⁵²

The sample, spotted as usual, is covered with a drop of concentrated sulfuric acid and developed with solvents such as hexane which are resistant to sulfuric acid.

Phenylhydrazone Formation²⁵²

The sample, spotted as usual, is covered with a drop of phenylhydrazine and then developed with a noncarbonyl solvent.

Semicarbazone Formation²⁵²

A 10 per cent solution of semicarbazide hydrochloride is neutralized with sodium hydroxide and spotted with the sample. The chromatogram is developed with a noncarbonyl solvent.

Hydrogenation¹⁸⁶

The spotting region of a layer is sprayed with a colloidal solution containing 1 to 2 per cent palladium in an electrolyte free solution.* The plate is dried in an oven, the sample is spotted in the region containing the palladium, and the layer is exposed to a hydrogen atmosphere in a vacuum desiccator. The plate is developed in the normal manner.

Irradiation³⁷¹

The spotted sample is irradiated with a suitable energy source in the desired atmosphere and developed in the normal manner.

Salt regeneration and bromination normally go to completion but the other reaction products are contaminated with starting materials which can be used, untreated, on the chromatograms as standards. The reactions listed certainly represent only a small portion of the possibilities for this technique.

* The solution is commercially available from Firma Dr. Th. Schuchardt, Ainmillerstr. 25, München 18, Germany.

CHAPTER 5

Choice of a Solvent System

INTRODUCTION

The type of solvent system chosen to make a given separation will depend primarily upon whether the chromatography is adsorption or partition. Adsorption chromatography is the simpler of the two because the solvents are completely miscible in one another and their ability to move a sample follows the normal eluotropic series. On the other hand, partition chromatography involves a selection of a two-phase system which will have desirable partition coefficients for the particular sample to be separated. Furthermore, partition chromatography can be of two types depending upon whether the stationary phase is polar (normal) or nonpolar (reversed phase). An even more complex situation involving various ionic species arises with ion-exchange chromatography on thin layers.

It is sometimes advantageous to add a small amount of some special reagent to developing systems to bring about a desired chemical reaction, to improve separations or to change the layer in some manner.

ADSORPTION CHROMATOGRAPHY

The normal worker will spend more time searching the literature for solvent systems to use in adsorption chromatography than he will in determining these systems by experimentation. This is particularly true since the exact adsorptive properties of layers tend to vary from one laboratory to another. The classic eluotropic series of Trappe,⁴⁰³ Strain³⁹³ and Knight and Groennings²⁰² apply to thin-layer chroma-

tography²³² and are given in Table 5.1. The more polar solvents producing the greatest migrations are at the bottom of the columns.

A preliminary study can be made in two ways. The first, devised by Izmailov and Shraiberg,¹⁶⁹ Crowe,⁶³ and further developed by Stahl,^{366,369,370} is the simpler. It involves the spotting of the sample in several places, an inch or so apart, on a single layer. Pure solvents of various polarities are deposited at the centers of these spots by means of a capillary and the outer edge of the solvent spot is noted. The layer is sprayed or visualized in some manner and the solvent which moves the sample about halfway between the origin and the outer edge is the best solvent. The second way to make a preliminary study is by running chromatograms with various solvents. In some laboratories, a series of chromatography jars containing certain key solvents like hexane, benzene, ether and methanol are kept ready for this purpose. In one of these ways, the ideal pure solvent or a pair of solvents whose properties "bracket" the material to be separated (one moving it less and one moving it more than desirable) are selected. In the latter case, various mixtures are investigated, or a gradient elution

TABLE 5.1. ELUOTROPIC SERIES OF SOLVENTS

| Trappe ⁴⁰³ | Strain ³⁹³ | Knight and Groennings ²⁰² |
|-----------------------|-----------------------------|--------------------------------------|
| Light petroleum | light petroleum 30-50° | heptane |
| Cyclohexane | light petroleum 50-70° | diisobutylene |
| Carbon tetrachloride | light petroleum 50-100° | benzene |
| Trichloroethylene | carbon tetrachloride | isopropyl chloride |
| Toluene | cyclohexane | isopropyl ether |
| Benzene | carbon disulfide | ethyl ether |
| Dichloromethane | anhydrous ethyl ether | ethyl acetate |
| Chloroform | anhydrous acetone | sec-butyl alcohol |
| Ethyl ether | benzene | ethyl alcohol |
| Ethyl acetate | toluene | water |
| Acetone | esters of organic acids | acetone |
| n-Propanol | 1,2-dichloroethane | methanol |
| Ethanol | alcohols | pyruvic acid |
| Methanol | water | |
| | pyridine | |
| | organic acids | |
| | mixtures of acids or bases, | |
| | water, alcohols, or pyr- | |
| | idine | |

system³³⁴ can be used. The gradient elution technique will be discussed more extensively in Chapter 6.

The author and others^{144,232} strongly recommend that developing mixtures be kept as simple as possible. In most situations, a mixture of two components will produce satisfactory results. For example, the author has separated a wide range of compounds such as glycosides, aglycones and nitrogen heterocyclics with mixtures of methanol and ether.

One should not become too concerned about whether the substance to be chromatographed is soluble in the solvent system. There appears to be no direct relation between *Rf* and solubility. This is probably because migration involves solution from a molecular or amorphous state while normal solubility requires the breakup of a crystal structure. Thus, sucrose and glucose can be moved readily on silica gel by methanol-ether solutions in which they are apparently insoluble.

NORMAL PARTITION CHROMATOGRAPHY

In this case, the substances to be separated (generally very polar compounds like sugars or amino acids) are caused to distribute themselves between a polar stationary phase (water, acetic acid, phenol or formamide) on the layer and a moving nonpolar phase. The nonpolar phase normally consists of two organic liquids having different polarities. Since the actual developing solution must contain a miscible mixture of all three of these components, it is likely that the literature will provide easier information than random experimentation.

The two liquids constituting the nonpolar phase provide the major point of variation for partition chromatography. Within the bounds of mutual solubility, their ratio can be varied to provide a greater or lesser dissolving power for the moving phase. The literature on paper chromatography provides at least a starting point in search for a system and is directly applicable when the layer consists of powdered cellulose³¹⁹ or Silica Gel G.⁴⁴ The literature summarized in Chapter 11 cites many solvent systems which have been used in thin-layer work.

REVERSED-PHASE PARTITION CHROMATOGRAPHY

In this case, the stationary phase is nonpolar and is usually a high boiling hydrocarbon or a Silicone liquid which has been applied to the layers are described in Chapter 3, p. 49. The moving, more polar phase, might be water, acetic acid, acetonitrile, alcohol, or some combination of these solvents. The method is useful for separating compounds of a homologous series such as the fatty acids or their derivatives. Table 5.2, taken mainly from Mangold²³² suggests several possible solvent systems.

TABLE 5.2. DEVELOPING SYSTEMS FOR REVERSED PHASE PARTITION CHROMATOGRAPHY*

| Solvents ¹ | Ratios, v/v | Stationary Phases ² | Lipid Classes Fractionated | Ref. |
|--|-------------|--------------------------------|--|------|
| Methanol | — | higher paraffins | carotenals | 449 |
| Acetic acid-water | 24/1 | undecane | fatty acids | 182 |
| — | 3/1 | Silicone oil | fatty acids and their methyl esters | 230 |
| — | 17/3 | Silicone oil | fatty acids and their methyl esters and aldehydic cores derived from lecithins | 230 |
| Acetic acid-acetonitrile | 1/1 | undecane | fatty acids | 182 |
| Acetic acid-acetonitrile-water | 2/14/5 | Silicone oil and squalane | methyl-esters of fatty acids | 230 |
| Methyl ethyl ketone-acetonitrile | 7/3 | higher paraffins | cholesteryl esters of fatty acids | 184 |
| Chloroform-methanol-water | 5/15/1 | undecane | diglycerides | 182 |
| Acetone-acetonitrile | 7/3 | undecane | triglycerides | 182 |
| Acetone-ethanol-water | 6/1/3 | Polyethylene | methyl esters of fatty acids | 232 |
| Acetic acid-formic acid-water ³ | 2/2/1 | Silicone oil | fatty acids saturated/unsaturated | 230 |
| Acetic acid-peracetic acid-water | 15/2/3 | Silicone oil | fatty acids saturated/unsaturated | 230 |

¹ All solvent mixtures must be saturated with the stationary phase.

² Silica Gel G served as a carrier for the stationary phases. Only polyethylene was applied directly to the plates.

³ Chromatographed at 4-6°. (All others at room temperature.)

* Reproduced from Mangold, *J. Am. Oil Chemists' Soc.*, **38**, 708 (1961) through the courtesy of the author and The American Oil Chemists' Society.

ION-EXCHANGE CHROMATOGRAPHY

The modified-cellulose ion exchangers DEAE and ECTEOLACelluloses have been used by Randerath^{316-318,320} for the separation of nucleotides. He used dilute hydrochloric acid (0.02 to 0.04 N) to develop his chromatograms. Determann, Wieland and Lüben⁸² used a gradient elution system, adding increasing amounts of sodium chloride to a phosphate buffer (*pH* 7.2), to separate enzymes and nucleotides on "Sephadex." Berger, Meyniel and Petit²⁹ used 1 M sodium nitrate to develop halogen ions on "Dowex 1" and acetic acid-methanol-acetone to separate fluorescein dyes on the same resin.

MISCELLANEOUS SPECIAL ADDITIVES

Several substances have been added to developing solutions for one reason or another. As mentioned (p. 58), bases,^{332,436} bromine^{186,295} and oxidizing agents²³⁰⁻²³² are added to bring about some reaction with the sample or some part of it. Seiler and Seiler³⁵⁹ have added small amounts (0.4 per cent) of the complexing agent, 2,5-hexanedi-one, to facilitate the separation of inorganic cations. Mistryukov²³⁴ added a small amount of decalin to developing solutions to be used on nonbound layers. The decalin increased the mechanical stability of the layer so that it could be visualized by spraying.

Small amounts of acidic or basic solvents are often added to developing systems to facilitate the separation of acids and bases, respectively, and to prevent "tailing" or smearing. "Tailing" is due, among other things, to the presence of more than one ionic species of the substances being chromatographed.²⁷⁴ Thus, Mangold²³² and others²⁶⁴ suggest the addition of 1 to 2 per cent of acetic acid to systems for developing chromatograms of fatty acids. In a similar manner, systems containing ammonia^{232,256} and diethylamine^{332,436} are used for separating nitrogenous lipids and other bases. These additives buffer the system and keep the material being separated exclusively in the non-ionic form.

CHAPTER 6

Development of Thin-Layer Chromatograms

INTRODUCTION

The development process, that is, the actual passing of solvents through thin layers to cause separations, is a major point of variation in thin-layer chromatography. Like the preparation of layers, it challenges the imagination of the laboratory worker and many ingenious techniques have resulted.

Thin-layer chromatography has been carried out in an ascending manner on vertical or near horizontal plates, in a descending manner on vertical plates, or in a linear or circular manner on horizontal plates. The development can be performed discontinuously, one pass or multiple pass, in a stepwise fashion with different solvents, continuously or by gradient elution methods. The layers can be scored in such a way that the chromatography is carried out in wedge or modified wedge-shaped areas. The process can be carried out in a two-dimensional fashion with different solvent systems being used in each direction. The layer or the sample may also be modified between two developments. These techniques will be discussed in detail in this chapter.

Finally, the various factors which influence the development or, more precisely, the R_f values resulting therefrom will be considered. These include the nature of the adsorbent and of the developing system, the nature and amount of the sample, the chamber saturation, the use of multicomponent solvent systems and the temperature. Some

methods of reporting *Rf* values and the causes and alleviation of tailing will be briefly discussed.

METHODS OF DEVELOPMENT

Ascending Chromatography

Most thin-layer chromatograms are developed by placing a glass plate, containing the sample on a bound, thin layer, in a vertical position in a closed, saturated system such that the bottom of the layer dips into the developing solvent. After the solvent ascends 10 to 15 cm, the plate is removed, dried, and visualized in some manner. Two or more layers can normally be processed in the same container provided that care is taken to completely saturate the system.

Kirchner, Miller and Keller¹⁹³ found that when water is a component of the developing system, the adsorbent layer tends to slide off of the plate below the solvent level. This problem was solved by standing the plate containing the layer on a cotton wad saturated with solvent. The author has observed a similar effect with very polar solvent systems, but found that even though the adsorbent below the liquid surface slides off, enough remains to carry the solvent into the layer satisfactorily.

While the plates are normally leaned against the side of the chromatographic jar or tank, several pieces of apparatus have been devised for holding them. A stainless-steel rack which will hold two large plates (20×20 cm) is available from Desaga-Brinkmann (p. 37), but a more versatile apparatus is available from Kensington Scientific Corp. (p. 36). This apparatus, pictured in Figure 6.1, is constructed from glass rods and will hold two plates (10×20 cm). It is suspended in a chromatographic chamber such that it can be raised or lowered without opening the chamber and thus allows the equilibration of a layer with the solvent before actual development. Such an equilibration step is recommended for partition chromatography.

When unbound layers are used (p. 46) they must be kept in a near-horizontal position (10 to 20° from horizontal) so that the adsorbent does not slide off. While other schemes have been devised,²⁵⁴ the simplest method for supporting these plates is that of Mottier and Potterat.²⁶⁹ As shown in Figure 6.2, the plates are propped in a large

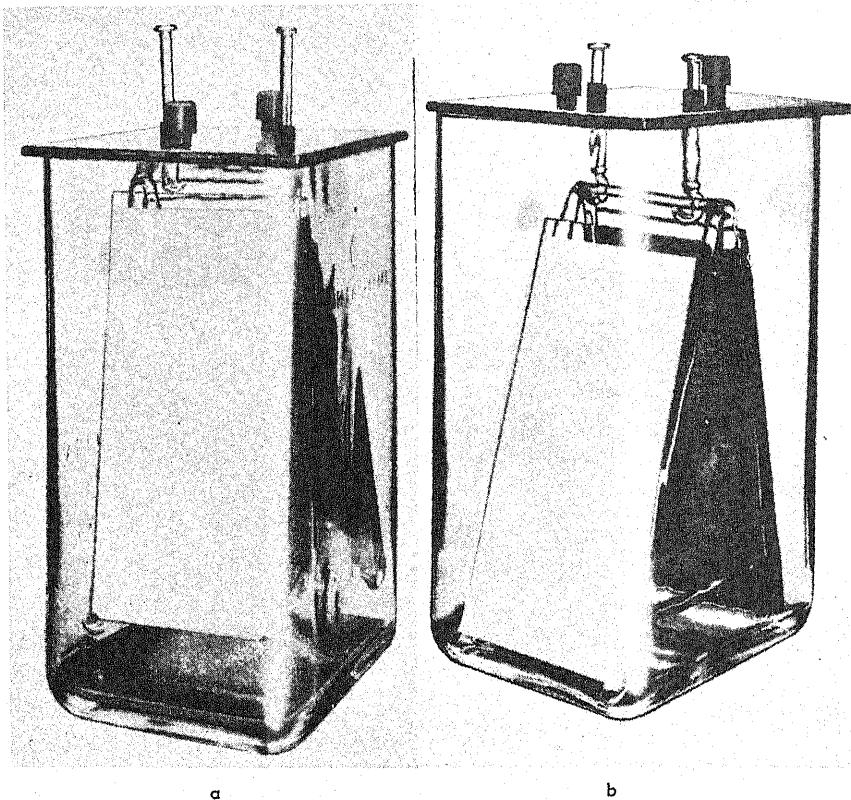


Figure 6.1. Chromatographic chamber containing a movable plate holding device which can be used to equilibrate thin-layer chromatograms prior to development. It is shown in a raised position, a, and a lowered position, b. (Reproduced through the courtesy of the Kensington Scientific Corp.)

Petri dish or crystallizing dish and the dish is tilted so that the solvent is confined to the portion touching the layer.

Descending Chromatography

The technique of descending thin-layer chromatography has essentially been worked out and applied by W. L. Stanley and his co-workers.^{383-385,388} They suspended 0.5 in. wide "chromatostrips"¹⁹³ coated with a silicic acid starch layer in an especially designed chamber as shown in Figure 6.3. The developing solvent is carried from the reservoir by a piece of coarse filter paper which is clipped to the top

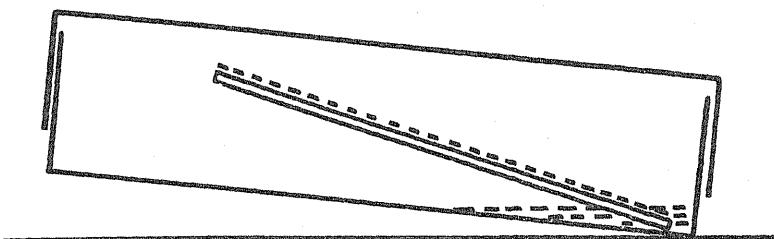


Figure 6.2. Development of a thin layer of unbound adsorbent. (Reproduced from Mottier and Potterat, *Anal. Chim. Acta*, 13, 46 (1955) through the courtesy of the authors and the Elsevier Publishing Co.)

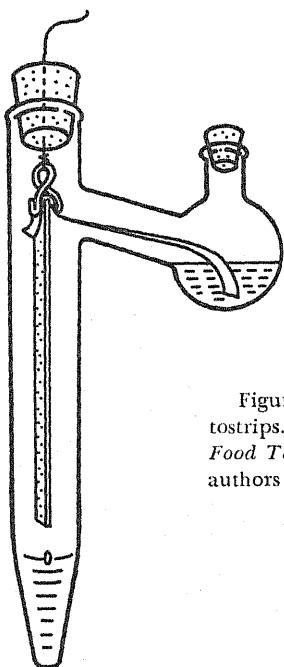


Figure 6.3. Descending chromatography on "chromatostrips." (Reproduced from Stanley, Ikeda and Cook, *Food Technol.*, 15, 381 (1961) through the courtesy of the authors and the Institute of Food Technologists.)

end of the layer. Such a system is advantageous in that it permits a continuous chromatography from which samples of eluent can be removed for analysis.³⁸⁵ Furthermore, it allows the use of relatively nonpolar solvents over a long time period and gives better separations.

Descending chromatography on wide plates containing the somewhat more fragile Silica Gel G layers has been carried out by Birkofe^r, Kaiser, Meyer-Stoll and Suppan³⁶ and by Zöllner and Wolfram.⁴⁵⁸ The apparatus of the latter authors is much the simpler of the two and is shown in Figure 6.4. The solvent is carried to the surface of the layer (facing down) by a piece of paper which also helps to saturate the atmosphere. The apparatus has an additional advantage in that a paper chromatography jar can easily be modified for the purpose.

Mistryukov²⁵⁵ has described an apparatus for the development, by descending chromatography, of unbound layers.

Horizontal Chromatography

Linear. Mistryukov²⁵⁴ has published a method for carrying out linear chromatography on completely horizontal unbound layers and the continuous chromatography of Brenner and Niederwieser^{45,46} as described below is on horizontal layers.

Circular. The original publication on bound layers by Meinhard and Hall²⁴⁶ involved circular chromatography of inorganic ions and since then, the technique has been used extensively.^{51,210,211,246,306,313,368} The sample is placed at the center of a layer and the solvent is fed into the center of the spot with a pipette^{210,211,246} or a cotton wick⁵¹ held in contact with the layer. The resulting chromatogram consists

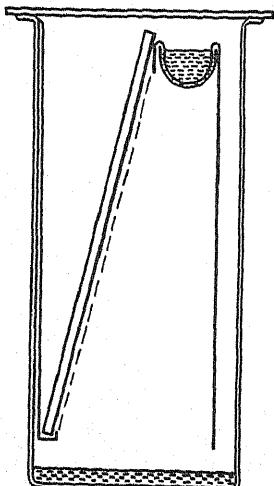


Figure 6.4. Descending chromatography on wide plates. The dotted line represents the layer. (Reproduced from Zöllner and Wolfram, *Klin. Wochschr.*, 40, 1098 (1962) through the courtesy of the authors and Springer Verlag).

of concentric rings. An alternate method which allows the comparison of several samples involves the placement of samples around and equidistant from a center point. Solvent is fed into the center point and the result consists of a series of chromatograms radiating from the center point. Figure 6.5 shows one arrangement for circular chromatography by Stahl.³⁶⁸ The apparatus requires a plate with a small hole in it.

Discontinuous Development

Single Pass. The vast majority of thin-layer chromatography has been carried out by allowing a single solvent system to pass through the layer one time. As discussed below, however, there is some reason to believe that better results could be obtained by different techniques.

Multiple Pass. Under the proper conditions, the development of a layer several times with the same solvent (with drying between developments) offers considerable advantage. The technique has been extensively used^{158,179,222,269,380,398} and has, in itself, been the subject of two important papers.

The first paper¹⁷⁵ dealt with paper chromatography, but should be equally applicable to thin layers. In it, Jeanes, Wise and Dimler state that the optimum number of passes, n_{\max} , can be predicted using Equation (6.1) where Rf_1 and Rf_2 are the values of individual com-

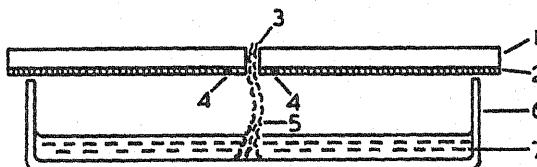


Figure 6.5. Circular thin-layer chromatography.

- | | |
|------------------------------------|----------------------|
| 1 glass plate (20×20 cm) | 5 cotton wick |
| 2 Silica Gel G layer (0.25) | 6 Petri dish cover |
| 3 2 mm hole | 7 developing solvent |
| 4 sample spots | |

(Reproduced from Stahl, *Parfüm. Kosmetik*, 39, 564 (1958) through the courtesy of the author and Dr. Alfred Huethig Verlag, Heidelberg, Germany).

$$n_{\max} = \frac{\log Rf_1 - \log Rf_2}{\log (1 - Rf_2) - \log (1 - Rf_1)} \quad (6.1)$$

pounds or Equation (6.2) where R_f is the average R_f value of the

$$n_{\max} = \frac{1}{R_f} - 1 \quad (6.2)$$

compounds. In the second paper¹⁰¹ which does discuss thin-layer chromatography, Thoma gives Equation (6.3) for the calculation (using the same symbols as 6.1 and 6.2). It is apparent from these equations

$$n_{\max} = \frac{-1}{\ln_e (1 - R_f)} \quad (6.3)$$

that systems with low R_f values (0.2 or less) can be greatly assisted by repeated developments while those systems with high R_f values cannot. Thoma was able to show that better separations were, in fact, obtained by several developments with a less polar solvent system than by one development with a more polar one.

Stepwise Development. Stahl and his co-workers^{309,378} devised a technique in which the layer was developed twice to differing heights with different solvent systems. Such a system is ideal when the sample contains a group of similar polar molecules and a group of similar nonpolar molecules. The chromatogram is first developed halfway (about 6 to 7 cm) with a polar system which deposits the nonpolar compounds at the solvent front about halfway up the layer and resolves the polar mixture in the bottom half of the chromatogram. The chromatogram is removed, dried and developed with a nonpolar solvent system. This system passes over the separated polar substances and resolves the nonpolar mixture in the top half of the chromatogram. This method is illustrated in Figure 6.6³⁷⁸ by the separation of a mixture of glycosides (polar) and aglycones (nonpolar). Similar schemes have been used by Billeter and Martius³⁵ for the separation of the K vitamins and by Mangold and Kammerer²³³ for the separation of lipid derivatives.

Weicker⁴³⁸ used still a third step for the separation of complex lipid mixtures. He first developed silica gel layers to 3 cm with propanol-ammonia (2:1). This carried the fatty acids, cholesterol and cholesterol esters to the solvent front and resolved lecithins and polar

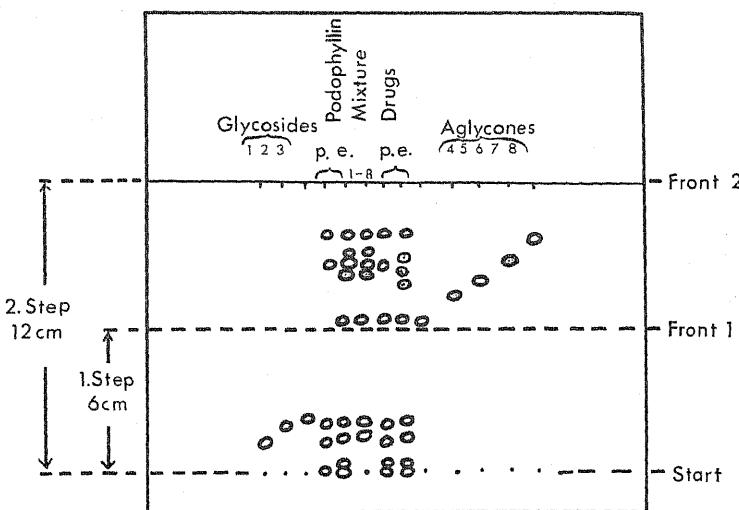


Figure 6.6. Thin-layer chromatography of *Podophyllum* components using a stepwise technique. 1. α -Peltatin- β -D-glucoside; 2. Podophyllotoxin glucoside; 3. β -Peltatin- β -D-glucoside; 4. 4'-Demethylpodophyllotoxin; 5. α -Peltatin; 6. Podophyllotoxin; 7. β -Peltatin; 8. 1-Deshydroxypodophyllotoxin. The plant extracts (marked podophyllin) and the drugs of two species of *Podophyllum*, *peltatum* L. (p) and *P. emodi* Wall (e) are shown along with a synthetic mixture of the eight compounds. The adsorbent was Silica Gel G and the solvent systems for steps 1 and 2 were 10% methanol in chloroform and 35% acetone in chloroform respectively. (Reproduced from Stahl and Kaltenbach, *J. Chromatog.*, 5, 548 (1961) through the courtesy of the authors and the Elsevier Publishing Co.)

lipids. A second development with chloroform-benzene (3:1) to 10 cm resolved the fatty acids and free cholesterol and carried the cholesterol esters to the solvent front. The layer was then rotated 180° and developed to a height of 4 cm with carbon tetrachloride to resolve cholesterol esters.

Still another variation⁴¹³ involves replacement of old adsorbent with new, and subsequent chromatography. Thus, in the separation of lipids, the crude mixture is developed first with ether-petroleum ether (5:95) on an unbound alumina layer. This moves the triglyceride fraction and the cholesterol esters. The adsorbent containing these components is removed and they are then assayed. The portion of the plate which contained these components is then cleaned

and a new layer added. Development with petroleum ether-ether-acetic acid (94.5:5:0.5), then moves and separates cholesterol, fatty acids and phospholipids on the new layer and they are subsequently removed and assayed.

Gradient Elution. Rybicka^{334,335} and Determann, Wieland, and Lüben⁸² have developed apparatus for gradient elution of thin-layer chromatograms, that is, for changing the solvent during the actual course of development. In this case a chromatography jar equipped with a magnetic stirrer is used. The chromatogram is dipped into one solvent (the less polar) in the bottom of the jar. Simultaneously, a second solvent (the more polar) is added from a burette which is connected to a glass tube extending through a hole in the top of the jar to the solvent surface. As the development and the addition continue, the properties of the solvent system slowly approach those of the added solvent. Using this technique, Rybicka separated glycerides^{334,335} on silica gel layers, Hofmann¹⁵³ separated proteins on hydroxylapatite and Determann, Wieland and Lüben⁸² separated enzymes and nucleotides on "DEAE-Sephadex."

Chromatography in Shaped Areas. Sometimes, layers are scored or divided with a sharp object into various shaped areas such as is shown in Figure 6.7. These shapes are such that the solvent enters through a narrow area of the layer and is forced to expand into the wider areas. The method was introduced by Mottier²⁶⁶ who used shape 6.7a in unbound alumina layers. Shapes 6.7b and 6.7c have been used by Stahl³⁶⁸ and Peereboom and Beekes²⁹⁵ and a wedge shape, 6.7d, is preferred by Prey, Berbalk and Kausz.³⁰⁷ The result of such a chromatogram is that the horizontal dimension of the spots is increased and the vertical dimension is decreased. The resulting narrow bands allow a greater number of compounds to be more completely separated than usual. An additional advantage of this technique is that small quantities of one component can be found in the presence of a large amount of another.³⁰⁷ The chromatogram 6.7c is the result of the separation of a mixture of one hundred parts of saccharose to one part of raffinose.

Two-Dimensional Chromatography. Sometimes, more components are in a mixture than will separate clearly on a one-dimensional strip. Or, perhaps, the mixture contains substances that differ from one

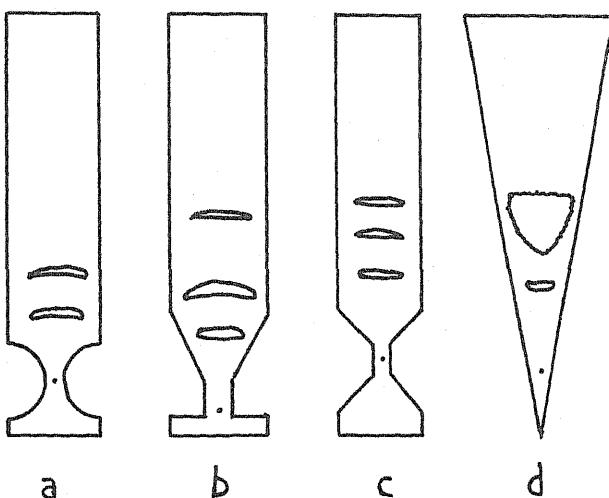


Figure 6.7. Chromatography in shaped areas according to Mottier,²⁰⁰ a, Stahl,²⁰¹ b, Peereboom and Beekes,²⁰⁵ c, and Prey, Berbalk and Kausz.²⁰⁷ d.

another so much that two solvent systems should be used. In these situations chromatography is often carried out in two directions which are perpendicular to one another. The general procedure is as follows. The sample is spotted in one corner of a square layer and developed so that the components are resolved along one edge of the plate. The chromatogram is then removed from the developing jar, dried and placed in a second solvent so that the previously formed component spots along the bottom of the plate are again submitted to chromatography. Such a system has been used extensively in thin-layer chromatography.^{21,44,46,87,182,184,193,221,269,317,352,371} The major point of variation in this technique is what is done to the solvent system or to the layer between the two developments.

Normally, two different solvent systems are used in the two developments so that the sample is exposed to the broadest possible conditions. The layer can be modified between the developments by dipping it in a hydrocarbon or a Silicone oil solution²³² to make a reversed phase chromatogram. The latter system has been especially useful in the lipid field where adsorption chromatography is used in one direction to separate the lipid classes and reversed phase chro-

matography is used in the second direction to resolve the classes. Anet⁵ has actually changed the adsorbent between developments. He first chromatographed 2,4-dinitrophenylhydrazones on Silica Gel G in one direction with toluene-ethyl acetate (3:1); then scraped off the adsorbent above the row of spots and replaced it with Aluminum Oxide G, and finally developed with the same solvent in the new adsorbent. The layers were made in each case with the modified⁴⁵² Camag apparatus (see p. 36).

Any of the modifying reactions listed in Chapter 3 (p. 57) can be carried out after the first development. Thus, hydrogenation,¹⁸⁶ bromination¹⁸⁶ or irradiation³⁷¹ sometimes modifies a mixture in such a way that the compounds which did not separate in the first development will do so in the second. This technique, called separation-reaction-separation by Stahl,³⁷¹ is clearly shown in the chromatogram in Figure 6.8 prepared by Stahl, where an irradiation reaction was carried out between separations. The chromatogram also shows the use of standards.

Continuous Development

Continuous development, or elution through a thin layer of adsorbent, offers two advantages. First, samples can be eluted off the end of the chromatogram, thus allowing the separation of mixtures containing components of widely differing polarities. Second, it allows the use of a relatively nonpolar solvent system over a long period of time. In this respect, it should be noted that a very polar developer tends, not only to move things too fast, but to override small differences between mixture components. Of course, with increased developing times, the spots diffuse more and separations become less sharp.

One method which has been used for this purpose (p. 68) is descending chromatography.^{385,388} In this technique, the solvent drips from the bottom of the layer into the chromatography chamber and can be collected easily. A commercial apparatus is available from Research Specialties Co. (see p. 40) for this purpose. The apparatus is similar to that shown in Figure 6.3 except that there is an opening in the bottom of the chamber.

A second approach for continuous development has been described

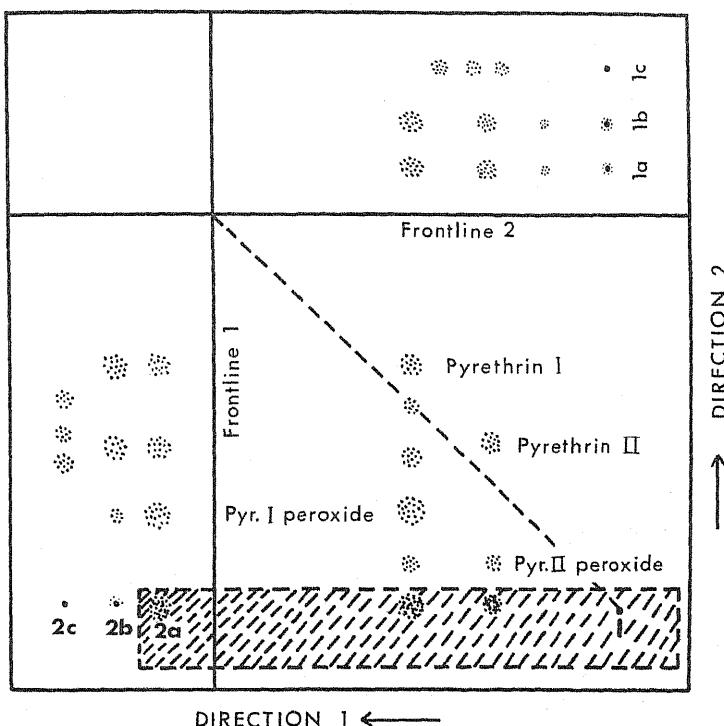


Figure 6.8. Thin-layer chromatography of pyrethrins by the separation-reaction separation-technique. The irradiation (dotted line area) was performed after the first separation. The same solvent system, 25% ethyl acetate in hexane, was used for both separations. The sample was initially placed at 1. Spots 1a, 1b and 2a and 2b are different concentrations of the starting mixture which will be developed in only one direction in order to orient the results of the two dimensional chromatogram. Spots 1c and 2c are a commercial (Desaga-Brinkmann) dye mixture of butter yellow, indophenol and Sudan Red G which is used to calibrate the layer and validate the R_f values given. The adsorbent was Silica Gel G. (Reproduced from Stahl, *Arch. Pharm.*, 293/65, 531 (1960) through the courtesy of the author and Verlag Chemie.)

by Brenner and Niederwieser.^{45,46} In this technique, the solvent is carried by a paper wick to one edge of a thin layer, passes through it and evaporates at the opposite edge. Samples cannot be taken from the end of the layer in this apparatus, which is illustrated in Figure 6.9. This has been used for the separation of amino acids⁹⁹ and for

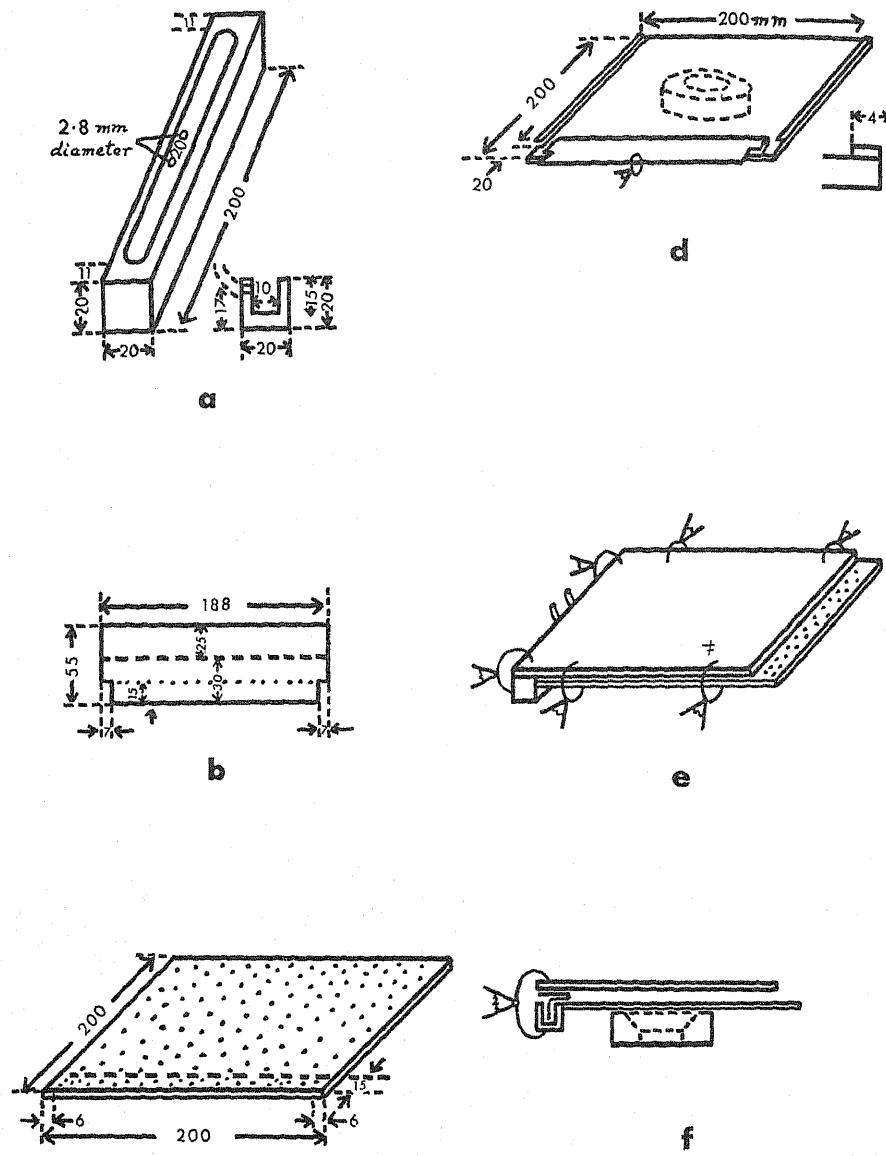


Figure 6.9. Brenner-Niederwieser apparatus for continuous, horizontal thin-layer chromatography. All measurements are in mm. Drawing a shows the steel solvent trough and the holes which feed solvent into it. Drawing b is the filter paper wick which carries the solvent to the layer. Drawing c shows a thin layer which has been spotted on a line 15 mm from the bottom and from which the adsorbent has been removed on the outermost 6 mm of the sides. Drawing d shows the layer in place on a cork ring with the wick in place and a detail of the edge strip which will support the cover plate. Drawing e shows a top view of the assembled apparatus and drawing f shows a side view (without the edge strips). In operation the sample is applied, the apparatus is assembled and the solvent is added. (Reproduced from Brenner and Niederwieser, *Experientia*, 17, 237 (1961) through the courtesy of the authors and Birkhaeuser Verlag).

preparative separations.¹⁵⁸ A commercial apparatus is available from Desaga-Brinkmann (see p. 37).

Essentially the same thing has been accomplished in a simpler fashion by developing in chambers without lids or by using a chamber shorter than the layer is long. In the latter case, a cover with a slit for the plate is used as shown in Figure 6.10a. The solvent evaporates after it emerges from the slit. The author has carried out continuous development by placing a piece of filter paper over the top of the plate as shown in Figure 6.10b. The filter paper is held in place with rubber bands or paper clips and leads the solvent over the top of the plate.

Layer Electrophoresis

Electrophoresis has been carried out on thin layers by Honegger¹⁵⁸ and by Pastuska and Trinks.²⁹¹ Honegger used Silica Gel G, Kieselguhr G and Aluminum Oxide G for the separation of amines and amino acids and combined the two processes of thin-layer chromatography and electrophoresis. That is, he separated a mixture by chromatography in one dimension and subjected the results to electrophoresis in the second dimension. Pastuska and Trinks investigated amino acids, phenols, amines and dyes. In both cases, the layer was

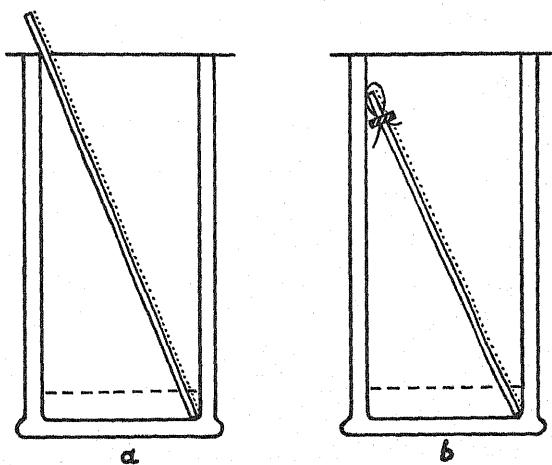


Figure 6.10. Simple arrangements for continuous thin-layer chromatography.

impregnated with an appropriate electrolyte and current was transmitted to the layer with filter paper moistened with electrolyte.

REPRODUCIBILITY OF *Rf* VALUES

One of the major disadvantages of thin-layer chromatography is that *Rf* values of substances are not exactly reproducible, at least from one laboratory to another. Furthermore, different samples of the same compound sometimes show slightly different behavior *even on the same layer*. These difficulties are more common in adsorption thin-layer chromatography and are probably traceable to the complexity of surface adsorption or to the fact that no two laboratories prepare layers in exactly the same manner. Many workers^{47,112,144,146,227,252,290} have commented extensively on this problem and their remarks will be summarized by considering the various factors which determine and affect *Rf* values.

Nature of the Adsorbent

The nature of the adsorbent and the thickness and moisture content of the layers are major factors in the determination of *Rf* values and have been discussed previously in this book. The use of standard, commercial adsorbents, layer thicknesses over 0.15 mm and consistent drying times will largely reduce the variations. It has been noted,⁴⁷ however, that there is an appreciable difference between batches of Silica Gel G. The worker should also remember that an activated layer becomes less active after exposure to a moist atmosphere for appreciable times.

Developing System

The nature of the developing system is, of course, a prime factor in any discussion of *Rf* values and should be controlled as closely as possible by the use of reagent grade solvents. Solvent mixtures should be made up frequently. This is especially true when very volatile solvents such as ether or pentane are being used. The mere opening of a chamber containing such a volatile solvent in a mixture can change the concentration appreciably.

Nature and Amount of Applied Sample

The nature of the sample, that is the properties of its components, is the third major factor in the determination of R_f values and is not controllable. The amount of sample does not have too much effect so long as it does not exceed the capacity of the system. Above this point, the R_f increases³⁵² or decreases and tailing or bearding results, depending upon whether the substance has a convex or a concave adsorption isotherm (see p. 82). The worker is urged to look at a given compound or mixture at several concentrations since the capacity of a layer will vary from one substance to another.⁴⁷

The R_f of a given substance may not be exactly the same in a mixture as it is in the pure state.²⁸⁸ If a given compound is suspected to be present in a mixture, this should be confirmed by chromatographing a pure sample against a mixture of the pure compound and the substance under investigation. The appropriate spot should increase in size if the compound is, in fact, a component.

Saturation of the Chamber

Care should be taken to saturate the chromatographic chamber with solvent as completely as possible before use.³⁶⁹ This is normally accomplished by partially lining the walls with filter paper which dips into the solvent and, when possible, by shaking the chamber before the introduction of the chromatogram.⁴⁷ Such saturation (called oversaturation by Stahl) will speed up development by about one-third,²³² will give lower, but more consistent R_f values and will produce straighter solvent fronts and rounder spots.

The so-called "edge effect,"^{42,369} that is, the difference in R_f values between the center and the edge of the plate, can largely be cured by complete chamber saturation if the layers have a fairly consistent thickness.

Multicomponent Developing Systems

There are two factors which should be watched when the developing system consists of several solvents. The first, as noted above, is the evaporation of the more volatile components and the second is the variation in concentration which will occur as the solvent front moves up the layer. To alleviate this latter situation, Brenner, Nieder-

wieser, Pataki and Fahmy⁴⁷ recommended that the ratio of the distance from the solvent level to the sample origin and the total distance traveled by the solvent front be kept constant when precise R_f values are being measured.

Temperature

Variations in temperature have little effect on thin-layer chromatography.⁴⁷ This is in contrast to the situation with paper chromatography where it is quite important. This is understandable where adsorption chromatography is concerned, but less so when the chromatography is partition.

REPORTING R_f VALUES

When reporting R_f values, it is a good idea to report the R_f values of some universally available substance or substances in the system used. The dye system of Stahl (butter yellow-indophenol-Sudan Red G)³⁶⁹ is readily available (from Desaga-Brinkmann) and is satisfactory for certain ranges of adsorption chromatography. As an alternate method, Dhont and de Rooy^{84,85} reported R_B values (sample migration/migration of butter yellow) for 2,4-dinitrophenylhydrazones and 3,5-dinitrobenzoates; Peereboom²⁹³ reports R_A values (sample migration/migration of dibutyl sebacate) for various esters used as plasticizers; and Lisboa and Diczfalussy²²¹ report R_{OB_1} values (sample migration/migration of estrone) for steroids.

TAILING

This bothersome phenomenon can normally be attributed to two factors, both of which have been discussed previously. The first, that is, the presence of various ionic species of acids and bases, can be alleviated by the addition of small quantities of acids and bases to the developing system (p. 65). The second factor is sample overloading such that one is working in the nonlinear portions of the isotherms of the substances being separated.* If the isotherms are convex, tail-

* This is discussed in considerable detail by Hagdahl on pages 63 and 64 and by Giddings and Keller on page 96 in Heftmann's book "Chromatography."¹⁴²

ing will occur in concentrations above the linear range. If they are concave, bearding or tails which precede the sample will result. In any case, the situation can be improved by working at lower concentrations.

CHROMATOGRAPHY CHAMBERS

Any wide-mouth jar or bottle which can be completely sealed will serve as a chromatography chamber. The ideal chamber is not too much larger than the plates which are to be developed so that chamber saturation is rapid and, further should have a fairly flat bottom so that the plate will be straight. Special chambers are available from all of the manufacturers of thin-layer equipment.

CHAPTER 7

Visualization

INTRODUCTION

The visualization of developed thin-layer chromatograms can be carried out in many ways. The method used in a specific case will depend upon the type of compounds to be visualized, the nature of the adsorbent and the purpose of the chromatogram. Most visualization methods involve a spray reagent, but other techniques are available.

The inorganic character of most thin layers permits the use of corrosive spray reagents such as sulfuric acid, nitric acid, chromic acid or mixtures of these. These acids will convert almost any organic compound to carbon and thereby serve as universal reagents. The applicability of such reagents constitutes one of the major advantages of thin-layer chromatography. In addition, those specific spray reagents which have been developed for paper chromatography and which do not involve a mechanical washing step can be used.

The majority of spray reagents are destructive in that they destroy or modify the materials which they visualize. In preparative thin-layer work or in quantitative work where the sample is to be eluted before assay, such destructive methods cannot be used. Techniques for surmounting this problem will be discussed in Chapters 9 and 10.

Visualization techniques involving the presence of phosphors or fluorescent substances in thin-layer adsorbents have been developed as well as methods depending upon the relative transparencies of adsorbent and adsorbed compounds when sprayed with the proper reagent. Finally, techniques of radiochemistry and bioassay have been applied to the problem of visualization.

CORROSIVE AND UNIVERSAL SPRAY REAGENTS

With the exception of iodine, these reagents are strong dehydrating and oxidizing systems which function by converting organic compounds to carbon. The reagents are sprayed on the chromatograms which are subsequently heated to about 100°, and the organic compounds appear as black spots. The system is not without flaws, however. When layers containing silver nitrate are sprayed with sulfuric acid,²⁰⁰ a very strong oxidizing system is produced which oxidizes the organic compounds to carbon dioxide. A second difficulty arises when the compound is very unreactive or volatilizes before it chars. In the case of camphor,¹⁹³ this difficulty was solved by spraying the chromatogram with concentrated sulfuric acid containing 5 per cent nitric acid. The sprayed chromatogram was placed, face down, on a glass cloth and heated on a hot plate registering approximately 500°. After the acid fumes ceased to come off, the glass plate was removed and the layer (which had come loose from the plate) was turned over with the glass cloth. The compounds showed as black spots. Such a volatility has been utilized by Baehler,¹⁰ who visualized chromatograms by subliming the resolved compounds out of the adsorbent layer on to a cooled plate held 1 mm above the heated layer (see p. 115).

Iodine, either in a spray or as a vapor, is an exceptionally fine universal reagent and has been used extensively (see Table 7.1). It appears to concentrate itself in the region containing an organic compound,^{219,244} thus producing a brown spot. The normal technique is to spray with 1% solution of iodine in methanol or to place the layer in a closed container with a few crystals of iodine. In either case, the spots fade rapidly and must be marked quickly. Under normal conditions, the reagent does not appear to modify the adsorbed substances.^{17,230,244} If one wishes more permanent spots, the layers can be sprayed with a 0.5 per cent solution of benzidine in absolute ethanol while the iodine spots are still visible.⁶

A number of universal reagents are given in Table 7.1.

SPECIFIC SPRAY REAGENTS

In general, paper chromatographic spray systems which do not require mechanical washing are applicable to thin layers. If, however,

TABLE 7.1. UNIVERSAL SPRAY REAGENTS USED IN
THIN-LAYER CHROMATOGRAPHY

| Reagent | Composition and Use | Ref. |
|--|---|--|
| 1. Conc. H_2SO_4 | Spray with acid and heat to 100–110°. | 193 and many others |
| 50% H_2SO_4 | Spray with reagent and heat to 200°. Observe in daylight and U.V. | 25, 26, 27, 262, 264, 310, 420 |
| 2% H_2SO_4 in H_2O -EtOH (1:1) | Spray with reagent and heat to 100°. | 221 |
| H_2SO_4 in acetic anhydride | Spray with H_2SO_4 -acetic anhydride (1:3) and heat. | 363, 368, 378 |
| 2. H_2SO_4 -KMnO ₄ | Sol. A: 0.5 g KMnO ₄ in 15 ml conc. H_2SO_4^1 | 19, 98 |
| | a. Spray with A. | |
| | b. Heat. | |
| 3. H_2SO_4 -Na ₂ Cr ₂ O ₇ | Sol. A: 3 g Na ₂ Cr ₂ O ₇ in 20 ml H ₂ O diluted with 10 ml conc. H_2SO_4 | 98 |
| | a. Spray with A. | |
| | b. Heat. | |
| H_2SO_4 -K ₂ Cr ₂ O ₇ | Spray with sat. sol. of K ₂ Cr ₂ O ₇ in conc. H_2SO_4 and heat. | 95 |
| H_2SO_4 -chromic acid | Spray with a sat. sol. of chromic acid in conc. H_2SO_4 and heat. | 232 |
| 4. H_2SO_4 -HNO ₃ | Spray with H_2SO_4 -HNO ₃ (1:1) and heat. | 110, |
| | Spray with 5% HNO ₃ in H_2SO_4 and heat. | 190, 191, 193, 252 |
| 5. HClO ₄ | Spray with 2% (or 25%) sol. and heat to 150°. | 247, 296 |
| | Allow 70% acid to diffuse into the layer at right angle to developing direction. | 70 |
| 6. I ₂ | Spray with 1% of I ₂ in MeOH. Place in closed chamber containing I ₂ crystals. | 119, 130, 183, 186, 230, 232, 234, 235, 244, 249, 292, 361, 397, 420 |
| I ₂ -benzidine | Spray I ₂ treated layer with 0.5% sol. of benzidine in absolute EtOH. | 6 |

¹ Large quantities of KMnO₄ can lead to the formation of explosive manganese heptoxide.

Silica Gel G layers are treated with dichlorodimethylsilane,¹⁸⁵ they are stable enough to be washed. The procedure is to place the layer in a vacuum desiccator with the silane and evacuate it to 300 mm for fifteen minutes. The layer is removed and allowed to stand for

thirty minutes. It is then stable enough to be dipped in aqueous solutions.

A number of specific spray reagents are given in Table 7.2. Table 7.3 is a cross reference for Table 7.2.

Explanation of Table 7.2

Reagent. The reagents are listed alphabetically according to the major component of the reagent or, on occasion, the name of the reagent. The minor components as well as some additional names are given in the cross reference, Table 7.3.

Preparation and Use. When several similar preparations have been used, the author has chosen one to put in the table. However, the references apply to all documented applications of the reagents.

The following abbreviations are used to conserve space:

| | | | |
|-------|----------------|------------|--------------------|
| HOAc | acetic acid | PrOH | <i>n</i> -propanol |
| NaOAc | sodium acetate | BuOH | <i>n</i> -butanol |
| EtOAc | ethyl acetate | pet. ether | petroleum ether |
| EtOH | ethanol | EtOEt | ethyl ether |
| MeOH | methanol | | |

Type of Compounds. The types of compounds given cover most, but not all, of the total uses of a given reagent.

Color. The colors are generally taken from a single paper and should not be taken too seriously.

References. The references apply to the main bibliography.

METHODS INVOLVING FLUORESCENCE AND PHOSPHORESCENCE

The preparation of fluorescent and phosphorescent thin layers by the incorporation of chemicals in the adsorbent has been discussed in Chapter 2. Several fluorescent reagents such as 2',7'-dichlorofluorescein (0.2 per cent in ethanol,²³²) fluorescein (0.04 to 0.004 per cent sodium salt in water,^{144,227,366}) Rhodamine B (0.05 per cent in water,¹⁶⁵) and morin (0.05 per cent in methanol³⁴¹) can also be sprayed on thin-layer chromatograms.

In any case, the treated layers cause compounds containing conjugated double bonds to show up as colored spots when viewed in an

Thin-Layer Chromatography

TABLE 7.2. SPRAY REAGENTS

| | Reagent | Preparation and Use | Type of Compounds | Color | Ref. |
|---|---|--|---|------------------|-----------------|
| 1 | Acid Violet-7B (6BN) | Sol. A: 1.5% aqueous sol. of reagent. Do not heat over 60° a. Spray with A b. Warm to 100° for 20 min. | cations of the alkali and alkaline earth metals | varies | 315, 358 |
| 2 | Aluminum chloride | Sol. A: 5% alcoholic sol. of reagent a. Spray with A b. Observe in daylight and U.V. | flavonoids | yellow spots | 286 |
| 3 | Ammonia vapor | Expose to vapor. | flavonol glycosides | | 126 |
| 4 | Ammonium molybdate-HClO ₄ | Sol. A: 3 g of ammonium molybdate in 25 ml H ₂ O, 30 ml of 0.1 N HCl and 15 ml 60% HClO ₄ a. Spray with A b. Heat to 110° for 15 min. | universal for lipids | blue-black spots | 157 |
| 5 | Ammonium molybdate-SnCl ₂ | Sol. A: 1% aqueous sol. of ammonium molybdate Sol. B: 1% SnCl ₂ in 10% HCl a. Spray with A b. Dry c. Spray with B d. Heat to 105° | phosphates (inorganic) | blue spots | 355 |
| 6 | Aniline phthalate | Sol. A: 0.93 g aniline and 1.66 g phthalic acid in 100 ml of <i>t</i> -BuOH saturated with H ₂ O ⁽³³⁾ a. Spray with A b. Heat to 105° for 10 min. | reducing sugars | various colors | 439 |
| 6 | Anisaldehyde in H ₂ SO ₄ and HOAc | Sol. A: 0.5 ml of reagent in 0.5 ml conc. H ₂ SO ₄ , 9 ml of 95% EtOH and a few drops of HOAc a. Spray with A b. Heat to 105° for 25 min. Sol. A: 0.5 ml of reagent in 50 ml of HOAc and 1 ml conc. H ₂ SO ₄ a. Spray with A b. Heat to 120° for 6 min. | carbohydrates steroids | various blues | 377, 441 247 |

| | | | | |
|----|---|--|--|---|
| 7 | <i>p</i> -Anisidine | Spray with an alcoholic sol. 0.1 M in both <i>p</i> -anisidine and phthalic acid | honeybee larval foods reducing sugars | 292 various colors 350 |
| 8 | <i>p</i> -Anisidine phthalate CCl_4 | Sol. A: 20% (also 12 and 16) sol. of reagent in CCl_4 a. Spray with A b. Heat to 120° c. Observe in daylight and U.V. | terpenoids, steroids and essential oils | 117, 171, 286, 299, 368, 394 |
| 9 | Antimony pentachloride in CHCl_3 | Sol. A : sat. sol. of reagent in alcohol-free CHCl_3 a. Spray with A b. Heat to 100° for 10 min. c. Observe in daylight and U.V. | steroids, steroid glycosides, aliphatic lipids, vitamin A and others | 13, 21, 67, 68, 128, 129, 141, 145, 171, 223, 247, 257, 287, 368, 371, 382, 392, 415, 416, 457, 460 |
| 10 | Antimony trichloride in CHCl_3 with acetic anhydride | Sol. A: sat. sol. of reagent in alcohol-free CHCl_3 , acetic anhydride (5:1) a. Spray with A b. Heat to 130° for 5-10 min. c. Observe in daylight and U.V. | steroids and steroid glycosides | 322 |
| | Antimony trichloride in HOAc | Sol. A: equal parts, by weight, of SbCl_3 and HOAc a. Spray with A b. Heat to 95° for 5 min. ³³ | steroids | 348 |
| | Antimony trichloride in CHCl_3 with SOCl_2 | Spray with a sat. sol. of reagent in alcohol-free $\text{CHCl}_3\text{-SOCl}_2$ (10:1) | Δ^4 -steroids | 145 |
| 11 | <i>Aqua regia</i> -ammonium molybdate-vanadyl chloride | Sol. A: <i>aqua regia</i> (3 parts conc. HCl and 1 part conc. HNO_3) Sol. B: 3 ml of 5% ammonium molybdate and 7 ml 5 N HCl diluted to 100 ml with acetone Sol. C: 2 g V_2O_5 and 20 ml conc. HCl boiled until blue green, then diluted to 37 ml with 6 N HCl and to 400 ml with H_2O ; 20 ml of this is then shaken with zinc powder until it becomes brown; decant and use immediately a. Spray with A, B and C consecutively | phosphorus in phosphates blue spots | 174 |

Thin-Layer Chromatography

TABLE 7.2.—Continued

| | Reagent | Preparation and Use | Type of Compounds | Color | Ref. |
|----|------------------------------|--|---|------------------------|-------------------------------------|
| 12 | Benzidine, diazotized | Sol. A: 0.1 to 0.085 M Na_2HPO_4 Sol. B: 0.18 g benzidine in 50 ml 0.5 N HCl Sol. C: 2 ml of B diluted with 2 ml 1% NaNO_2 , allowed to lose its color (3–5 min), treated with 2 ml of 5% urea and finally diluted with 14 ml H_2O a. Spray with A b. Spray with C _{14a} | phenols which couple | violet | 288, 290, 291 127, 197, 427, 432 |
| 13 | Bial's reagent | Sol. A: 40.7 ml conc. HCl mixed with 0.1 g orcinol, 1% FeCl_3 and diluted with H_2O to 50 ml a. Place in HCl atmosphere at 80° for 1.5 hr. b. Spray with A c. Place in HCl atmosphere at 80° until reaction occurs | glycolipids | yellow | 221 |
| 14 | Boule reaction | a. Expose to NH_3 vapor b. Expose to nitrogen dioxide (from Cu and HNO_3) | estrogens with phenol groups | yellow | 42, 105, 106, 107, 193 |
| 15 | Bromoresol green | Spray with a 0.3% sol. of reagent in $\text{H}_2\text{O}-\text{MeOH}$ (20:80) containing 8 drops of 30% NaOH per 100 ml. | carboxylic acids | yellow spots on green | 357, 200, 201 |
| 16 | Bromoresol purple | Spray with a 0.1% sol. of reagent in EtOH made just basic with NH_4OH or NaOH | a. halogen ions, except F^- b. dicarboxylic acids | yellow spots on purple | 302 |
| 17 | Bromophenol blue-citric acid | Sol. A: 0.5 g of bromophenol blue and 0.2 g of citric acid in 100 ml H_2O a. Spray with A | aliphatic carboxylic acids | yellow spots on blue | 302 |
| 18 | Bromthymol blue | I. <i>alkaline</i> Spray with a sol. of 40 mg of reagent in 0.01 N NaOH (100 ml) II. <i>buffered</i> Spray with a sol. of 50 mg of reagent and 1.25 g of boric acid in 8 ml of 1 N NaOH and 112 ml H_2O | universal for lipids | yellow spots on blue | 134, 173, 174, 208, 459 |

| | | | | | |
|----|---|--|--|----------------------|----------|
| 19 | Ceric sulfate-trichloroacetic acid | Sol. A: 0.1 g Ce(SO ₄) ₂ suspended in H ₂ O; add 1 g of trichloroacetic acid; boil and add, drop by drop, 1.84 ml of conc. H ₂ SO ₄ until it becomes clear a. Spray with A b. Heat to 110° ^a | tocopherols | various colors | 354 |
| 20 | Chloramine T-HCl-NH ₃ | Sol. A: 10% reagent in water Sol. B: 1 N HCl a. Spray with A b. Spray with B c. Heat to drive off Cl ₂ d. Expose to NH ₃ ³⁵ | caffeine | rose red | 117 |
| 21 | Chlorine-KI-starch | a. Expose to Cl ₂ for 0.5 hr. b. Drive off excess Cl ₂ by waving in air c. Spray with sol. of KI and starch Sol. A: 160 mg toluidine in 30 ml HOAc; add 1 g KI and make up to 500 ml. a. Expose to Cl ₂ for 5-10 min. b. Allow to stand in air 3-5 min. c. Spray with A ³⁵ | esters of carbobenzoxy-amino acids | blue spots | 456 |
| | Chlorine-toluidine-KI | Sol. A: 160 mg toluidine in 30 ml HOAc; add 1 g KI and make up to 500 ml. a. Expose to Cl ₂ for 5-10 min. b. Allow to stand in air 3-5 min. c. Spray with A ³⁵ | 3-phenyl-2-thiohydantoins of amino acids | 46 | |
| 22 | Chromotropic acid in H ₂ SO ₄ | Sol. A: 10% aqueous sodium 1,8-dihydroxynaphthalene-3,6-disulfonate-23 N H ₂ SO ₄ (1:5) a. Spray with A b. Heat to 105° for 30 min. | methyleneendiox group | blue | 32 |
| 23 | Chlorosulfonic acid-HOAc | Sol. A: CISO ₃ H-HOAc (1:2) a. Spray with A b. Heat to 130° for 5 min. c. Observe in U.V. | triterpenes and cardiac aglycones | violet-brown | 405, 406 |
| 24 | Copper acetate-dithioxamide on stabilized layers | Sol. A: 20 ml of sat. aqueous copper acetate diluted to 1000 ml Sol. B: 0.1% dithioxamide in EtOH a. Dip layers in H ₂ O b. Dip layers in A for 10 min. c. Wash with H ₂ O for 30 min. d. Dip in B for 10 min. e. Wash in H ₂ O | fatty acids | green spots on white | 180 |

Thin-Layer Chromatography

TABLE 7.2—Continued

| | Reagent | Preparation and Use | Type of Compounds | Color | Ref. |
|----|---|--|--|------------------------|---|
| 25 | Cyanogen bromide-henzidine | Sol. A: 0.05% benzidine in 2 N HOAc a. Expose to CNBr vapor b. Spray with A | nicotinic acid derivatives | red to brown | 117 |
| 26 | α -Cyclodextrin-I ₂ | Sol. A: 1.0% sol. of reagent in H ₂ O-EtOH (70:30) a. Spray with A b. Expose to I ₂ | monochain lipids | white spots on purple | 230, 231, 232 |
| 27 | <i>o</i> -Dianisidine | Spray with a 1% sol. of reagent in HOAc | aldehydes and ketones | yellow-brown | 193, 252, 328, 368 |
| 28 | Dibromo-R-fluorescein | Sol. A: 0.2% sol. of reagent in EtOH a. Spray with A b. Observe in U.V. | glycerides | yellow fluorescent | 16 |
| 29 | 2',7'-Dichlorofluorescein | Sol. A: 0.2% sol. of reagent in 96% EtOH a. Spray with A b. Observe in U.V. | nonpolar lipids, saturated and unsaturated | green spots on purple | 8, 28, 120, 230, 232, 234, 235, 236, 264, 323, 334, 363, 420, 423 |
| 30 | 2,6-Dichloroquinone-chlorimide-NH ₃ | Sol. A: 0.1% sol. of reagent in EtOH a. Spray with A b. Expose to NH ₃ | Vitamin B ₆ | blue spots | 116, 117 |
| 31 | 2,6-Dichloroquinone-chlorimide-borax | Sol. A: 2% quinone in EtOH Sol. B: 2% borax in water a. Spray with A and B consecutively | phosphate esters | blue spots | 293 |
| 32 | N,N-Dimethyl- <i>p</i> -phenylenediamine-2HCl-NaOMe-irradiation | Sol. A: 0.5 g of diamine in 100 ml MeOH with 1 g Na a. Spray with A b. Moisten with H ₂ O c. Expose to U.V. without filter | chlorinated hydrocarbons | green-violet spots | 23 |
| 33 | <i>p</i> -Dimethylaminobenzaldehyde in HCl (van Urk reagent) | Sol. A: 0.1 to 1% reagent in 30 ml of conc. HCl and 50 ml 96% EtOH a. Spray heavily with reagent b. Spray lightly with <i>agua regia</i> (for alkaloids) | sulfonamides alkaloids (indole) | 452 blue rose spots | 376 |
| | <i>p</i> -Dimethylaminobenzaldehyde in H ₂ SO ₄ | Spray with 1% sol. of reagent in 2% sol. of H ₂ SO ₄ in EtOH | a. essential oils b. morphine | | 287, 437 |

| | | | | | |
|------|--|--|---|---------------------------------|--|
| 33 | 2,4-Dinitrophenylhydrazine (2,4-DNPH) | Spray with a 0.5% sol. of reagent in 2 N HCl | aldehydes and ketones | yellow to red spots | 2, 13, 105, 106, 107, 165, 171, 192, 287, 323, 326, 328, 368, 376 |
| 33a. | DPNH-reduced diphospho-pyridine nucleotide | | enzymes | | 82 |
| 34 | Diphenylamine | Sol. A: 1% sol. of reagent in EtOH a. Spray with A. b. Expose to U.V. | nitramine explosives | blue spots | 216 |
| | Diphenylamine in HCl and HOAc | Sol. A: 10% sol. of amine in EtOH a. Dilute 20 ml of A with 100 ml conc. HCl and 80 ml HOAc b. Spray with diluted A. c. Cover with another plate and heat to 110° for 30-40 min. | glycolipids | blue | 157, 174 |
| 35 | Diphenylboric acid- β -amino-ethyl ester | Sol. A: 1% of reagent in MeOH a. Spray with A. b. Observe in U.V. | flavonols, coumarins and their glycosides | varies | 379 |
| 36 | s-Diphenylcarbazone | Spray with a 0.1% sol. of reagent in 95% EtOH | acetoxymethoxymercury adducts of unsaturated lipids | purple to blue spots on rose | 177, 232, 233 |
| 36a. | Diphenylthiocarbazone | Sol. A: 0.01% of reagent in CHCl ₃ a. Spray with A | organotin derivatives | orange-red | 411 |
| 37 | Dragendorff's reagent | I. Sol. A: 1.7 g of basic bismuth nitrate in 100 ml of H ₂ O-HOAc (80:20) Sol. B: 40 g KI in 100 ml H ₂ O a. Spray with sol. made from 5 ml of A, 5 ml of B, 20 g of acetic acid and 70 ml H ₂ O II. Sol. A: Warm 2.6 g of bismuth carbonate and 7 g of NaI in 25 ml of HOAc. After 12 hr, filter sol. and dilute 20 ml of the filtrate with 8 ml EtOAc Sol. B: 10 ml of A diluted with 25 ml HOAc and 60 ml EtOAc a. Spray with B b. Colors can be enhanced by spraying with 0.1 N H ₂ SO ₄ ³¹⁵ | alkaloids and choline containing compounds | orange | 88, 157, 188, 227, 273, 332, 344, 427, 430, 444 |

Thin-Layer Chromatography

TABLE 7.2.—Continued

| | Reagent | Preparation and Use | Type of Compounds | Color | Ref. |
|----|--|--|---|-------------------------|--|
| 38 | Ehrlich reagent | Sol. A: 1% sol. of <i>p</i> -dimethylaminobenzaldehyde in 95% EtOH a. Spray with A b. Place in HCl atmosphere for 3–5 min. | a. tryptophane metabolites b. gangliosides | various colors | 87, 208 |
| 39 | a. Echt blue salt B (tetraazotized di- <i>o</i> -enisidine) b. Echt blue salt BB (diazo-tized 1-amino-4-benzoyl-amido-2,5-dioethoxyben-zene). c. Echt red salt B (diazotized 5-nitro-2-aminoanisole) | Sol. A: 5% aqueous sol. of reagent a. Spray with A b. Spray with 0.1 N NaOH | phenols and amines, which couple | | 288 |
| 40 | Ferric chloride Ferric chloride in HOAc | Spray with a 1% aqueous sol. of reagent Sol. A: Equal volumes of 5% aqueous FeCl ₃ and 2 N HOAc a. Spray with A | phenols pyrazolones | various colors | 287, 292 |
| | Ferric chloride-K ₄ Fe(CN) ₆ | Sol. A: 1 volume 5% aqueous K ₄ Fe(CN) ₆ , 2 volumes 10% aqueous FeCl ₃ and 3 volumes H ₂ O a. Spray with A | phenacetin phenols chlorinated hydrocarbons | blue 117 98 43 | 227 |
| 41 | Ferrous ammonium sulfate-potassium thiocyanate | Spray with a mixture of the two aqueous solu-tions | peroxides | | 242 |
| 42 | Fluorescein | Sol. A: 0.04% aqueous sol. of sodium fluorescein a. Spray with A b. Observe in U.V. | conjugated double-bond systems | | 82, 227 |
| | Fluorescein-Br ₂ | Sol. A: 0.04% aqueous sol. of sodium fluorescein a. Spray with A b. Observe in U.V. for conjugated systems c. Expose to Br ₂ d. Observe in U.V. for unsaturates | unsaturated compounds | yellow spots on pink | 105, 106, 107, 171, 190, 191, 192, 193, 232, 252, 328, 365, 368 |

| | | | | |
|----|--------------------------------------|---|----------------------------|----------------|
| 43 | Folin-Ciocalteau reagent | Sol. A : Mix 10 g sodium tungstate, 2.5 g of sodium molybdate and 70 ml H_2O . Add, consecutively, 5 ml 85% H_3PO_4 and 10 ml conc. HCl . Reflux for 10 hr and add 15 g Li_2SO_4 , 5 ml H_2O and 1 dp Br_2 . Dilute to 100 ml with H_2O . Reagent should not be green. Sol. B: 20% aqueous Na_2CO_3 a. Spray with B b. Dilute A with 3 volumes H_2O and spray ³⁷⁵ | a. phenols b. estrogens | 163, 221, 380 |
| 44 | Fuchsin- H_2SO_4 | Sol. A: 1 g parafuchsin is dissolved in 700 ml H_2O and 50 ml 2 N HCl, diluted to 1 l with H_2O and allowed to stand overnight with occasional shaking. a. Add 1% $HgCl_2$ to A b. Spray with reagent ⁴⁸ | plasmalegens | 134 |
| 45 | Hydrazine sulfate in HCl | Spray with a 1% sol. of reagent in 1 N HCl | aromatic aldehydes | Yellow |
| 46 | Hydrogen chloride | Hold over conc. HCl | chalcones | red |
| 47 | Hydroxamic acid-ferric ion | Sol. A: equal volumes of 12.5% NaOH and 5% hydroxylamine, both in H_2O Sol. B: acetic acid Sol. C: 10% $FeCl_3$ in H_2O a. Spray with A b. Allow to stand for 10 min (or heat to 100° for 10 min) c. Spray with B and C consecutively | esters of all types | varies |
| 48 | 8-Hydroxyquinalidine-NH ₃ | Sol. A: 0.5% sol. of reagent in 60% EtOH a. Expose to NH_3 b. Spray with A c. Observe in U.V. | inorganic cations | various colors |
| | | | | 359, 360 |

Thin-Layer Chromatography

TABLE 7.2—Continued

| | Reagent | Preparation and Use | Type of Compounds | Color | Ref. |
|----|--|---|---------------------------|----------------|------|
| 49 | Iodine-potassium iodoplatinate | Sol. A: 0.5% sol. of I ₂ in CHCl ₃ Sol. B: equal volumes of 1.1% aqueous KI and 0.135% aqueous chloroplatinic acid a. Spray with A and B alternating | nitrogen-containing drugs | yellow | 279 |
| | Iodine in KI and HOAc | Sol. A: aqueous sol. which contains 5% I ₂ and 10% KI a. Dilute 2 ml of A with 3 ml H ₂ O and 5 ml 2 N HOAc and spray with diluted sol. | alkaloids and steroids | 347, 227 | |
| | Iodine-benzidine | Sol. A: 5% benzidine in EtOH a. Expose to I ₂ vapor b. Spray with A | glycerides | blue | 6 |
| 50 | Iodine azide | Sol. A: 3.5 g of sodium azide in 100 ml of 0.1 N I ₂ (reagent is explosive when dry ⁴⁵) a. Spray with A | penicillins | yellow | 103 |
| 51 | Isatin in H ₂ SO ₄ | Sol. A: 0.4% sol. of reagent in conc. H ₂ SO ₄ a. Spray with A b. Heat to 120° | thiophene derivatives | various colors | 65 |
| 52 | isonicotinic acid hydrazide | Sol. A: 4 g isonicotinic acid hydrazide and 5 ml conc. HCl in 1 l H ₂ O a. Spray with A b. Allow to stand 3-16 hr. c. Observe in U.V. and daylight | steroids | | 414 |
| 53 | Kedde reagent | Sol. A: 2% sol. of 3,5-dinitrobenzoic acid in MeOH Sol. B: 2 N KOH a. Mix equal volumes of A and B just before spraying b. Spray with mixture | cardiac glycosides | | 223 |

| | | | | | |
|----|--|--|---|---------------------------------------|--|
| 54 | Morin (2',3,4',5,7-pentahydroxyflavone) | Sol. A: 0.05 to 0.005% sol. of reagent in MeOH a. Spray with A. b. Heat to 100° for 2 min. (optional) c. Observe in U.V. | a. N-blocked amino acids b. conjugated double-bond systems | yellow | 52, 341 |
| 55 | N-(1-Naphthyl)ethylene diamine·2 HCl (diazonized) | Sol. A: 1 N HCl Sol. B: 5% NaNO ₂ Sol. C: 0.1% reagent in EtOH a. Spray with A, B, and C (consecutively) | sulfonamides | reddish purple | 196 |
| 56 | Naphthoresorcinol in H ₃ PO ₄ | Sol. A: 100 ml of 0.2% sol. of reagent in EtOH and 10 ml H ₃ PO ₄ a. Spray with A. b. Heat to 105° for 5-10 min. | carbohydrates | various colors depending upon type | 120, 289, 307, 308 |
| 57 | Ninhydrin | I. Sol. A: 95 ml of 0.2% reagent in BuOH plus 5 ml of 10% aqueous HOAc a. Spray with A. b. Heat to 120-150° for 10-15 min. II. Sol. A: 50 ml of 0.2% sol. of reagent in abs. EtOH mixed with 10 ml of HOAc and 2 ml of 2,4,6-collidine Sol. B: 0.1% Cu(NO ₃) ₂ ·3H ₂ O in abs. EtOH a. Spray with A-B (50:3) mixed just before use b. Heat to 105° | a. amino acids b. aminophosphonates c. amino sugars | blue | 44, 95, 99, 116, 117, 134, 156, 292, 424, 438, 450 |
| 58 | Osmium tetroxide | Expose to reagent vapors (caution, poison) in closed chamber | cmpds. with double bonds | brown to black | 459 |
| 59 | Palladium chloride in HCl | Spray with a 0.5% sol. of reagent in dilute HCl | thiophosphate insecticides | yellow spots on brown | 23 |
| 60 | Periodic acid-HClO ₄ -V ₂ O ₅ | Sol. A: 10 g H ₄ IO ₆ in 100 ml of 70% HClO ₄ and a few crystals of V ₂ O ₅ a. Spray carefully dried layer with A Sol. A: 0.5% HIO ₄ in 90% HOAc Sol. B: 30% sol. of sodium pyrosulfite-3 N HCl (1:1) (both kept at 0°) Sol. C: 5 ml of 10% sodium pyrosulfite, 85 ml H ₂ O and 0.2 g tuchsin. Allow sol. to stand 12 hr, treat with carbon and filter a. Spray with A, B, and C consecutively b. Heat to 90° for 15 min. | thiophosphate esters glycerides | blue | 304 |
| | Periodic acid-Schiff's reagent | | | violet | 174 |

Thin-Layer Chromatography

TABLE 7.2.—Continued

| | Reagent | Preparation and Use | Type of Compounds | Color | Ref. |
|----|---|---|--|------------|--|
| 61 | Phosphomolybdc acid | Sol. A: 1.5% (also 5 and 10%) sol. of reagent in EtOH or EtOH-EtOEt (1:1) a. Spray with A b. Heat to 150° for 5 min. | a. bile acids b. glycerides c. unsaturated fatty acids d. many others | blue | 13, 115, 152, 154, 155, 157, 179, 181, 182, 183, 184, 186, 238, 249, 293, 295, 362 |
| | Phosphomolybdc acid-SnCl ₂ | Sol. A: 1% sol. of acid in CHCl ₃ -EtOH (1:1) Sol. B: 1% SnCl ₂ in 2 N HCl a. Spray with A b. Dry and dip in B | phospholipids | blue | 185 |
| 62 | Phosphomolybdc acid-NH ₃ | Sol. A: 20% sol. of acid in EtOH a. Spray with A b. Dry for 2-3 min and expose to NH ₃ | tocopherols | blue | 354 |
| 63 | Photophotungstic acid | Sol. A: 10% sol. of acid in EtOH a. Spray with A b. Heat to 100° for 5-10 min. c. Observe in U.V. | cholesterol esters | | 249 |
| 64 | Phosphoric acid | a. Spray with 50-70% acid b. Heat to 100° for 10-30 min. c. Observe in U.V. | pregnanetriols and other steroids | | 247, 391, 414 |
| 65 | <i>ortho</i> -Phosphoric acid-phosphomolybdc acid | Sol. A: 40% <i>ortho</i> -phosphoric acid Sol. B: 5% sol. of phosphomolybdc acid a. Spray with A b. Heat to 110° for 7 min. c. Observe in U.V. d. Spray with B e. Heat to 110° for 2 min. | steroids | blue-green | 433, 434, 435 |

| | | | | |
|----|---|---|---|--|
| 66 | Potassium hydroxide | Sol. A: 5% KOH-acetone (2:1) a. Spray with A b. Heat to 100° c. Cool and spray (optional) with $\text{HNO}_3\text{-H}_2\text{SO}_4$ (1:1) | aromatic nitro compounds and amines | 110 |
| | Potassium hydroxide-urea | Sol. A: 1 N KOH in EtOH Sol. B: 50% aqueous sol. of urea a. Spray with A b. Heat to 60° for 15 min. (citrates are yellow) c. Spray with B d. Observe in U.V. | citrates | 293 |
| 67 | Potassium iodide | Spray with saturated aqueous sol. Sol. A: 2% sol. of KI in H ₂ O a. Spray with A b. Observe colors c. Expose to NH ₃ d. Expose to H ₂ S | peroxides copper series of inorganic cations | 242 359 |
| | Potassium iodide-HOAc-Na ₂ SO ₃ -starch | Sol. A: fresh sol. of 5 ml of 4% KI, 20 ml HOAc and a few drops of 1% Na ₂ SO ₃ Sol. B: 1% aqueous sol. of starch a. Spray with A and B consecutively | peroxides | 371 |
| 68 | Potassium iodoplatinate | Sol. A: mix 45 ml of 10% KI with 5 ml of 5% platinum chloride and dilute to 100 ml a. Spray with A | alkaloids and vitamins | various colors 57, 116, 117, 279, 285, 426 |
| 69 | Potassium permanganate (alkaline) | Spray with 0.25% aqueous sol. of reagent Sol. A: equal portions of 1% aqueous KMnO ₄ and 2% aqueous NaClO ₃ a. Spray with A b. Note color after 1-2 hr. | diterpenoids phenols and plasticizers (esters) | 213 290, 293 |
| | Potassium permanganate-HOAc | Spray with mixture of equal volumes of 0.1 N KMnO ₄ and 2 N HOAc | oxidizable materials | 227 |
| | Potassium permanganate-Fe(No ₃) ₃ | Sol. A: 0.5% aqueous sol. of KMnO ₄ Sol. B: 1% aqueous sol. of Fe(No ₃) ₃ a. Spray with A and B consecutively | phenols | 340 |

TABLE 7.2—Continued

| | Reagent | Preparation and Use | Type of Compounds | Color | Ref. |
|----|---|---|-------------------------|----------------------|--|
| 70 | Procházka reagent | Sol. A: 10 ml of 35% formaldehyde, 10 ml of 25% HCl and 20 ml 96% EtOH a. Spray with A b. Heat to 100° c. Observe in daylight and U.V. | indole derivatives | yellow | 376 |
| 71 | Pyridyl-azo-naphthalol | Spray with a 0.25% sol. of reagent in EtOH | uranium ion | | 360 |
| 72 | Resorcinol-SnCl ₂ -H ₂ SO ₄ -KOH | Sol. A: 20% resorcinol in EtOH with added SnCl ₂ Sol. B: 4 N H ₂ SO ₄ Sol. C: 40% aqueous KOH a. Spray with A b. Heat to 150° for 10 min. c. Spray with B d. Heat to 120° for 20 min. e. Spray with C | esters and plasticizers | various colors | 293 |
| 73 | Rhodamine B | Spray with a 0.05% (or 0.5%) sol. of reagent in EtOH | lipids | purple spots on rose | 19, 165, 181, 182, 183, 232, 427, 430, 448 |
| | Rhodamine B-KOH | Sol. A: 0.05% aqueous sol. of reagent Sol. B: 10 N KOH a. Spray with A and B consecutively | glycerides | bright spots on rose | 6 |
| 74 | Rhodamine 6 G | Sol. A: 0.05% aqueous sol. of reagent a. Spray with A b. Observe in U.V. | lipids | | 459 |
| 75 | Silver nitrate-NH ₂ NaOMe | Sol. A: 0.3% AgNO ₃ in MeOH Sol. B: MeOH saturated with NH ₃ Sol. C: 7% Na in MeOH a. Mix 5 parts of A, 1 part of B and 2 parts of C b. Spray with mixture c. Note color after few min. ^{61a} | acyl sugar | brown | 75 |
| 76 | Silver nitrate-NH ₄ OH-fluorescein | Sol. A: 1% sol. of AgNO ₃ made basic with NH ₄ OH Sol. B: 0.1% sol. fluorescein in EtOH a. Spray with A and B consecutively | halogen ions | | 357 |

| | | Spray with a 1% aqueous sol. of reagent | ferrocenes | blue | 345 |
|----|--|--|--|---------------|-----------------------------|
| 77 | Sodium periodate | Sol. A: 4 parts of 2% aqueous NaIO_4 and 1 part of 1% KMnO_4 in 2% aqueous Na_2CO_3 a. Spray with A ^{51a} | sugars | | 454 |
| 78 | Sodium periodate-K MnO_4 | Sodium periodate-ethylene glycol-thiobarbituric acid Sol. A: 0.02 M NaIO_4 , Sol. B: ethylene glycol-acetone-H 2SO_4 (50:50:0.3) Sol. C: 6% aqueous sol. of 2-thiobarbituric acid sodium salt a. Spray with A, B, and C consecutively. ^{45b} | neuraminic acid sialic acid derivatives | red | 127 |
| 79 | Stannous chloride-HCl | Spray with a 10% sol. of SnCl_2 in conc. HCl | aromatic nitro compounds | blue | 110 |
| 80 | Sudan Black B on stabilized layers | Sol. A: 0.1% sol. of reagent in EtOH-H 2O (1:1) a. Dip in A b. Wash with EtOH-H 2O (1:1). | saturated glycerides | blue | 180 |
| 81 | Sulfanilic acid, diazotized (Fauly's reagent) | Sol. A: 0.5 g of sulfanilic acid and 0.5 g of KNO_2 in 100 ml of 1 N HCl I. a. Spray with A b. Spray with 1 N NaOH II. a. Spray with conc. KOH in MeOH b. Spray with A | phenols and aromatic amines which will couple | yellow-orange | 11, 72, 74, 82, 396, 426 |
| 82 | Tetrazolium blue or 3,3'-(3,3'-dimethoxy-4,4'-biphenylene)-bis[2,5-di-phenyl-2H-tetrazolium chloride] ¹ | I. Reducing steroids Sol. A: 1% sol. in EtOH Sol. B: Mix just before using 2 ml of A, 5 ml of H 2O and 3 ml of 4 N NaOH a. Spray with A II. Δ^4 -3-oxosteroids Sol. A: 90 ml 2.7 N NaOH and 0.1 ml of sol. A above. a. Spray with A ^{51a} | corticoids | | 1, 243 |
| 83 | 2,3,5-triphenyltetrazolium chloride-NaOH | Sol. A: Mix just before use one volume of 4% sol. of reagent in MeOH and one volume of 1 N NaOH a. Spray with A b. Heat to 110° for 5-10 min. ³⁵ | a. steroids b. reducing sugars c. Glycosides | red spots | 30, 247, 414 |

TABLE 7.2—Continued

| | Reagent | Preparation and Use | Type of Compounds | Color | Ref. |
|----|---|---|---|---|-------------------|
| 84 | Trichloroacetic acid | Sol. A: 4% sol. of reagent in CHCl ₃ a. Spray with A b. Allow to stand | menthofuran K-strophanthin (in U.V.) cardenolides | pink reddish-brown | 19 223 |
| | Trichloroacetic acid-chloramine-T | Sol. A: Mix just before use 3 volumes of a 25% sol. of acid in EtOH with 2 volumes of 3% chloramine-T sol. a. Spray with A b. Heat to 100° for 10 min. c. Observe in daylight and U.V. | | | 90, 223, 322 |
| 85 | Trinitrobenzene | | azulenes | | 108 |
| 86 | Uranium acetate | Spray with a 1% sol. of reagent in H ₂ O | flavonoids | brown | 286 |
| 87 | Vanillin-H ₂ SO ₄ | Sol. A: 1% sol. of reagent in conc. H ₂ SO ₄ a. Spray with A. b. Observe in daylight and U.V. | terpenoids | various colors | 49, 167, 168, 368 |
| | Vanillin-H ₃ PO ₄ | Sol. A: 1% sol. of reagent in 50% H ₃ PO ₄ a. Spray with A. b. Heat to 120° for 10-20 min. | steroids | | 247, 336, 338 |
| 88 | Water on Silica Gel G | a. Spray thoroughly b. Observe in reflected light | alkaloids, steroids, sugar acetates and others | opaque areas on semi-transparent background | 115, 128, 398 |
| 89 | Zwicker's reagent | Sol. A: 0.5% sol. of cobalt acetate in MeOH Sol. B: 0.5% sol. of lithium hydroxide in MeOH a. Spray with A and B consecutively | barbiturates | | 227 |

TABLE 7.3. CROSS REFERENCES TO SPECIFIC SPRAY REAGENTS

| Reagent or Name | Item in 7.2 | Reagent or Name | Item in 7.2 |
|---|-------------|-------------------------|-------------|
| Acetic anhydride | 10 | Iodine | 26 |
| Benzidine | 25 | Lithium hydroxide | 89 |
| Bismuth nitrate | 37 | Pauly's reagent | 81 |
| Bismuth carbonate | 37 | Perchloric acid | 4 |
| Borax | 30 | Potassium ferricyanide | 40 |
| Bromine | 42 | Potassium iodide | 21 |
| Chloramine-T | 84 | Potassium iodoplatinate | 49 |
| Cobalt acetate | 89 | Potassium permanganate | 78 |
| 2,4,6-Collidine | 57 | Potassium thiocyanate | 41 |
| <i>p</i> -Dimethylaminobenzal-dehyde | 38 | Pyruvic acid | 33a |
| 3,5-Dinitrobenzoic acid | 53 | Schiff's reagent | 60 |
| Diphospho-pyridine nucleotide (reduced) | 33a | Sodium molybdate | 43 |
| Dithiooxamide | 24 | Sodium tungstate | 43 |
| Dithizone | 36a | Stannous chloride | 4, 72 |
| Ferric chloride | 47 | Starch | 21 |
| Fluorescein | 76 | Thiobarbituric acid | 78 |
| Formaldehyde | 70 | Thionyl chloride | 10 |
| Hydrogen sulfide | 67 | Toluidine | 21 |
| Hydroxylamine-HCl | 47 | Trichloroacetic acid | 19 |
| | | Urea | 66 |
| | | van Urk's reagent | 32 |

ultraviolet light.²³² If fluorescein sprayed layers are then treated with bromine vapor, the fluorescein is converted to eosin except where unsaturated compounds were present. The result is that the unsaturated compound shows up as a yellow spot on a pink background. It is best viewed in ultraviolet light.^{227,232,366}

TRANSPARENCY METHODS

If a developed layer is sprayed with or dipped into a liquid of the correct refractive index, the adsorbent and the compounds to be visualized will have relatively different transparencies and can be distinguished when held in a correct angle with respect to light. In this manner, spots or bands can be located without destruction and with great accuracy. The most commonly used system of this type is water on silica gel,^{115,128,398} in which the bands or spots stand out as semiopaque areas on a translucent background. The method would obviously fail if the adsorbed materials were water soluble.

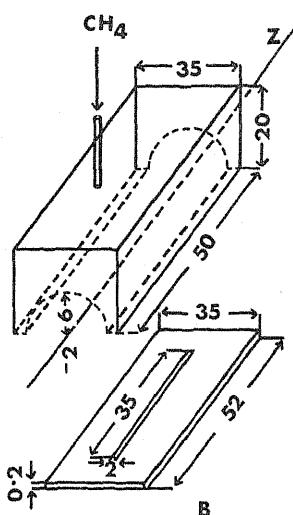


Figure 7.1. Gas flow counter with an extremely flat aperture plate (B) for measurement of weak β -emitters on thin-layer chromatograms. The aperture plate is attached to the counter tube without projecting parts. Z = counter wire (30μ diameter). The measurements are given in mm. (Reproduced from Schulze and Wenzel, *Angew. Chem. Intern. Ed. Engl.*, 1, 580 (1962) through the courtesy of the authors and the publishers, Verlag Chemie and Academic Press.)

In a variation of this technique, Kaufmann, Makus and Khoe¹⁸⁵ dipped Silica Gel G layers (which had been stabilized with dichlorodimethylsilane) in a mixture of ethanol-water (2:8) so that triglyceride spots became transparent. Hefendehl¹⁴¹ sprayed already visualized Silica Gel G layers with a paraffin-ether mixture (1:1). The paraffin-impregnated layers became transparent after being stored in a desiccator for a few hours and the colored spots were easier to photograph.

RADIOACTIVITY METHODS

Chromatograms of radioactive compounds can be visualized by making X-ray prints.^{20,232,234a} The layers are directly exposed to "No-Screen Medical X-Ray Safety Film"** for times varying from a few hours to a week. The films are developed with "Supermix Developer"*** for 4 to 6 min. and fixed for 30 min. with "Acid Fixer." These methods have been applied mostly to lipids, although radioactive halides have also been detected by radioactivity.²⁹

Schulze and Wenzel³⁴⁰ developed a method for continuous auto-

* Eastman Kodak Company, Rochester 3, N.Y.

** General Electric X-Ray Dept., Milwaukee 1, Wisc.

matic measurement of radioactivity distribution of weak β -emitters on thin layers. Accurate measurements of C¹⁴ and tritium-containing compounds were made by passing the developed layer slowly under the aperture of the especially designed gas-flow counter shown in Figure 7.1.* The gas was methane. The method was also used for locating radioactive materials in preparative thin layer chromatography.

BIOASSAY METHODS

The developed chromatogram is covered with an agar plate containing an appropriate microorganism in good health. The spots which contain antibiotic inhibit the microorganisms and can thereby be detected. Thus, Nicolaus, Coronelli and Binaghi^{275,276} located a number of penicillins, tetracyclines and rifomycins using *Sarcina lutea* and *Bacillus subtilis* in the presence of triphenyltetrazolium salts. The sensitivity was 0.01 to 0.1 γ . The macrolides have been determined³³ using *Bacillus subtilis* as the revealing organism.

* A commercial model is available from Laboratorium Prof. Dr. Berthold, Wildbad (Schwarzwald) Germany.

CHAPTER 8

Documentation of Results

INTRODUCTION

Thin-layer chromatograms are normally too fragile for preservation as such and several techniques have been devised for making a permanent record of them. In general, these are to trace them or draw them in a laboratory book, to photograph or photocopy them, or to imbed them in a paraffin layer or plastic film of some type. The latter two, more exact, documentation methods offer the additional advantage that the photograph or films can often be quantitized.

PHOTOGRAPHY

The most positive way to record thin-layer chromatograms is to photograph them.^{46,137,141,223,262,273,328,352,416} Chromatograms which are visible in normal light can be photographed with any available camera system. Hefendehl¹⁴¹ found that better pictures resulted when the chromatograms were made semitransparent after normal visualization procedures (see p. 104).

Thin-layer chromatograms can also be copied with an office letter-copying device such as the "Zerox 914,"*¹⁴⁸ "Photorapid"**¹²¹ or the "Copease" apparatus.†²³² A process using a direct positive blueprinting paper, Driprint HC (F speed)‡ has been suggested.⁹⁶ In some

* Manufactured by Zerox Corp., Rochester 3, N.Y.

** Manufactured by Bürogeräte, A. G., Zürich, Switzerland, Pronto model with Gevalcopy paper GS, 8½ × 11 in.

† Manufactured by Copease Corp., 425 Park Avenue, New York, N.Y.

‡ E. Dietzgen Co., 407 10th Street, N.W., Washington, D.C.

cases^{96,121} the layer is covered with cellophane film before photography to protect both the layer and the photocopying apparatus. It has also been suggested¹⁴⁸ that better copies will result if the spots are outlined with a stylus or even completely removed before duplication.

Sylvania F8T5/BLB, "Blacklite" blue tubes* and a yellow filter are recommended by Bürke and Bolliger²³² for black and white photography in ultraviolet light. Lukas has made photographs in ultraviolet light using an ultraviolet filter,²²³ and Hansbury, Langham and Ott¹³⁷ have devised a method using a "Polaroid" system for the same purpose.

The use of X-ray film for recording chromatograms of radioactive substances was discussed in Chapter 7 (p. 104).

METHODS OF LAYER PRESERVATION

Meinhard and Hall²⁴⁶ pressed a piece of "Scotch" or cellophane tape on thin layers to remove a portion of the layer. The tape was then placed directly in a laboratory book. The applicability of this technique is governed by the visualization procedure and the spray reagents used.

Mottier^{265,267} and Machata²²⁷ prepared semipermanent records of chromatograms by imbedding the layer in paraffin. In the case of unbound adsorbents, paraffin is applied to the plate as though it were another developing solvent, that is, by dipping the lower part of the layer in the melted paraffin and allowing it to rise by capillary action.^{265,267} The bound layers are impregnated by dipping them in melted paraffin.²²⁷ Semipermanent records can also be made by covering the layer with another glass plate and thus preserving it as a sandwich between two plates.^{227,232} The sandwiches are sealed by taping them around the edges.

A more permanent preparation can be made by imbedding the adsorbent layer in a plastic film which can be peeled off of the glass plates and preserved as such. This is accomplished by spraying the chromatogram with a 4 per cent collodion solution containing 7.5

* Sylvania Electric Products, Inc., 500 5th Ave., New York, N.Y.

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per cent glycerol,¹⁸ aqueous dispersions containing 10 to 15 per cent of polyacrylic acid esters, polyvinylidene chloride and polyvinyl propionate,²¹⁸ Fisher Label Glaze,*²³² or "Neatan" (Brinkmann, see p. 37).

* Fisher Scientific Company, 633 Greenwich Street, New York, N.Y.

CHAPTER 9

Preparative Thin-Layer Chromatography

INTRODUCTION

The preparative separation of reasonable amounts of a mixture (10 to 1000 mg) on thin layers has been a relatively neglected aspect of thin-layer chromatography. The high capacities of the adsorbents (when used in adsorption chromatography), the high degrees of resolution obtained, and the over-all simplicity of the method make it a near ideal tool for the organic chemist. The technique itself involves the mechanical steps of normal thin-layer chromatography (Chapters 2 to 8) with slight modifications.

Thus, the layers are thicker (0.5 to 2 mm compared with 0.25 mm) than normal so that the maximum amount of material can be separated at once. The sample is not applied in spots but in a narrow band and developed in such a manner that the resolved components lie in bands. These bands are located by some nondestructive visualization technique; the adsorbent containing a single component is removed from the band area and the sample is removed from the adsorbent by elution with an appropriate solvent. Quantities ranging from 10 to 250 mg can be separated on a single chromatogram by this technique and a number of chromatograms can conveniently be carried out simultaneously.

The development of this aspect of thin-layer chromatography has been severely hampered by the lack of methods for sample application and for the nondestructive visualization of the developed chro-

matogram. The sample must be applied in large quantities to a relatively thin band without undue destruction of the rather fragile layer. It now appears that satisfactory, though not ideal, solutions have been found for these problems.

At least two attempts have been made to apply the concepts of thin-layer chromatography to large-scale separations. These involve chromatography in cast blocks or "chromatobars"²⁵⁰ of plaster-of-Paris-bound adsorbent and chromatography in horizontal cellophane tubes filled with adsorbents.⁸⁶ A third similar technique is chromatography on layers of cellulose one inch thick packed between glass plates.¹³⁵ While each of these methods has certainly been successful in itself, the author believes that they are generally more laborious and inferior to the technique outlined below.

The various aspects of preparative thin-layer chromatography will be dealt with in the order and in the same manner as has been done in the preceding chapters for the general technique. Numerous examples of preparative work are cited as such in the tables of Chapter 11.

ADSORBENTS

Both bound and unbound adsorbents can be used with no special treatment other than perhaps a prepurification step (see pp. 19 and 48).

PREPARATION OF LAYERS

For the sake of convenience, most preparative thin-layer chromatography has been carried out on the usual square two-dimensional plates (20 × 20 cm or 8 × 8 in.). Larger plates could, of course, be used to separate larger quantities, but their use becomes awkward above this size and commercial apparatus is not available for preparation of the layers.

The adsorbent slurries have usually been made in the normal manner although Honegger suggests¹⁵⁸ the use of somewhat less water and a little more binder for very thick layers (see Table 3.2). Ritter and Meyer³²⁹ found the optimum layer thickness to be about 1 mm

although Honegger¹⁵⁸ gives a somewhat wider range of 1 to 3 mm. The very thick layers tend to crack and there is an appreciable difference between behavior on the open side of the layer and the side next to the glass. Layers up to 2 mm thick can be made with the adjustable Stahl applicator (see p. 37) and layers up to 5 mm thick can be made with the Camag apparatus (see p. 36).¹⁵⁸

The layers are best allowed to dry at room temperature for several hours before final activation. This prevents casehardening and subsequent cracking. It is further recommended that, as the original Desaga instructions suggest, the layers be dried in a vertical position rather than horizontal.

SAMPLE APPLICATION

Sample application is made by applying the sample, in an appropriate solvent, in a thin band about 2 cm from one edge of the layer. A smooth application can be effected using the mechanical devices of Morgan²⁵⁸ or of Ritter and Meyer³²⁹ as discussed in Chapter 4. Alternately, the sample can be applied from a syringe moved manually along the layer. The operation is best carried out on a heated metal block^{223,329} to evaporate the solvent. Honegger¹⁵⁸ applied the sample in a V-shaped trough cut in the layer.

The amount of sample applied will depend upon the specific mixture to be separated, the adsorbent involved and upon the type of chromatography. Much larger samples can be separated by adsorption than by partition chromatography. Mangold states²³² that up to 50 mg can be separated by adsorption chromatography on a layer of Morgan²⁵⁸ or of Ritter and Meyer³²⁹ as discussed in Chapter 4. Meyer³²⁹ separated 75 mg on a plate of the same size with a 1-mm-thick layer. Honegger states¹⁵⁸ that 5 to 25 mg can be separated on such a 1-mm layer and Černý, Joska and Lábler⁵² separated 20 to 100 mg of steroids on a layer of unbound alumina 20 × 10 cm and 1 mm thick. The author considers it "safe" to apply 50 mg of any mixture to a layer 20 × 20 cm and 1 mm thick and has applied as much as 300 mg of an easily separated mixture. For partition chromatography, only about one-tenth of these sample sizes should be used. Mangold²³² suggests 5 mg on a 20 × 20 cm layer 0.25 mm thick.

CHOICE OF DEVELOPMENT SOLVENTS

The solvents for preparative chromatography are the same as those used for normal thin layers.

DEVELOPMENT

The development of preparative chromatograms is carried out in the normal way with the following precautions. If several chromatograms are developed simultaneously, special care must be taken to saturate the chamber. The author prefers to develop such chromatograms with adsorbent turned toward the chamber wall and close to, but not touching, a layer of filter paper on the wall. If several chromatograms are to be developed in sequence in the same chamber, it should be remembered that thick layers remove more solvent than thin layers and the composition of the solvent may change appreciably.

All of the tricks of development discussed in Chapter 6 except those involving two-dimensional chromatography are applicable. Multiple-step and continuous development are particularly useful.¹⁵⁸

VISUALIZATION

In the ideal case, the bands on a developed chromatogram will be colored or can be seen in ultraviolet light.^{52,224} Bands containing conjugated double-bond systems can be located by using an adsorbent containing phosphors or a fluorescent additive as discussed in Chapter 2 (p. 21). Sometimes, small amounts of fluorescent or colored impurities will be present in a sample which will allow the orientation of a preparative plate with respect to a small plate (preferably with the same layer thickness) which has been visualized in a normal destructive manner. Schulze and Wenzel³⁴⁹ used their gas-flow counter to find bands of compounds containing C¹⁴ and tritium labels.

Mistryukov²⁵⁵ describes the use of "paper prints" to locate zones. Narrow paper strips (5 to 6 mm) are moistened and pressed against an unbound layer. After removal, the strips contain a layer of adsorbent and can be visualized with any spray reagent.

Iodine appears to be a good visualization reagent for most lipids²³⁰

since it does not affect them appreciably. Gritter and Albers¹²⁸ have located steroids and other types of compounds by spraying the layer with water as discussed in Chapter 7 (p. 103). When all of these methods fail, an edge spray technique can be used. The preparative layer is covered with a glass plate except for 2 to 3 cm along one vertical edge and a line of adsorbent is scraped off along the edge of the cover plate to isolate the narrow edge strip.³²⁹ The edge is then sprayed with an appropriate reagent, and the results are extrapolated across the layer.^{230,333,420, and others}

ELUTION OF COMPOUNDS

Most workers simply scrape the adsorbent containing the desired components from the glass plate with a spatula and elute it with an appropriate solvent. However, this is difficult when using unbound layers and two gadgets have been devised for the purpose. Both operate on the vacuum cleaner principle. The first, as shown in Figure 9.1, is a piece of glass tubing with a constriction and holds a cotton wad to catch the adsorbent. It was devised by Mottier^{267,269} and has the advantage that it can be used as an elution tube and no sample is lost in transfer. The Ritter and Meyer apparatus,³²⁹ as shown in Figure 9.2, is more suitable for large quantities. The adsorbent is sucked into a Soxhlet extractor thimble from which it is eluted by extraction.

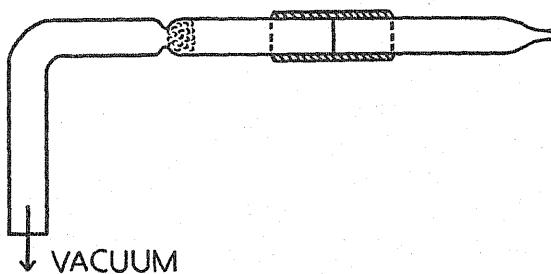


Figure 9.1. Mottier gadget for removing adsorbent from a thin layer. (Reproduced from Mottier, *Mitt. Gebiete Lebensm. Hyg.*, 49, 454 (1958) through the courtesy of the author and the Eidgenoessische Drucksachen und Materialzentrale, Bern, Switzerland.)

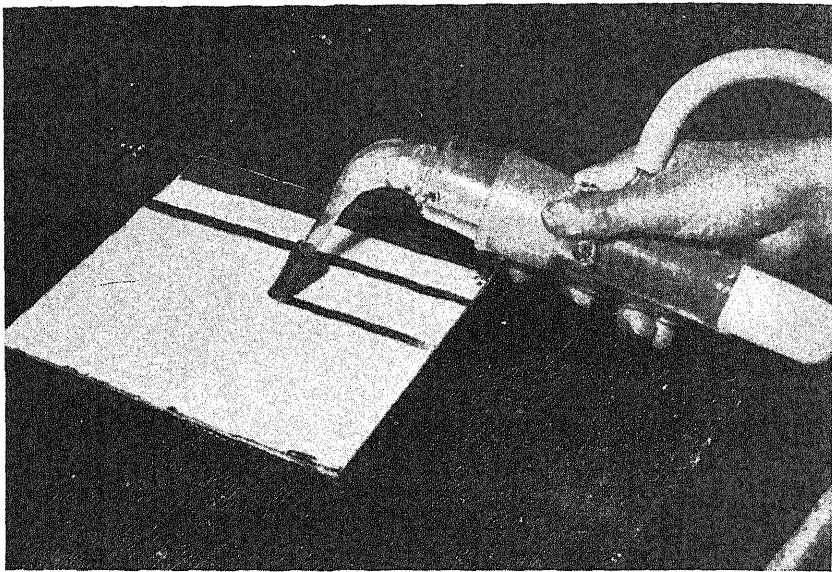


Figure 9.2. Ritter and Meyer apparatus for removing adsorbent from a thin layer.
(Reproduced from Ritter and Meyer, *Nature*, 193, 941 (1962) through the courtesy of the authors and Macmillan and Co., Ltd.)

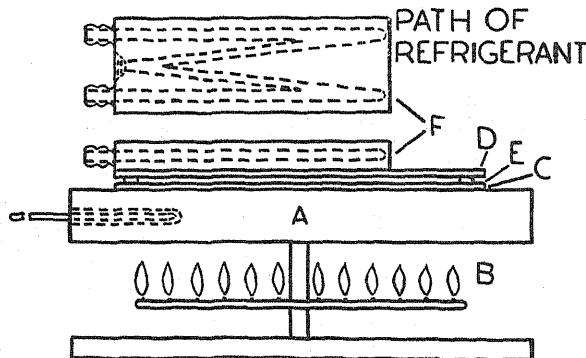


Figure 9.3. The apparatus of Baehler for direct sublimation out of thin layers. A refrigerant is passed through the metallic block (F) which rests on cover plate (D). The 1 mm spacer separates the cover plate from plate (C) which bears the thin layer. Block A is a piece of metal containing a thermometer which is heated by burners (B). (Reproduced from Bachler, *Helv. Chim. Acta*, 45, 309 (1962) through the courtesy of the author and Verlag Helvetica Chimica Acta, Basel, Switzerland.)

The ideal elution solvent is one which moves the sample with the solvent front on a thin layer.²³² Aqueous eluents cannot be used with plaster-of-Paris-bound adsorbents because they will dissolve appreciable amounts of calcium sulfate. It is essentially impossible to avoid the solution of small amounts of silica gel in any eluting solvent. If the eluent is evaporated to dryness and taken up in a fresh solvent, preferably less polar than the original, most of the adsorbent will remain on the sides of the flask.

Sometimes, the substances on a chromatogram can be removed by direct sublimation to a cooled plate held about 1 mm over the adsorbent layer. Bachler¹⁰ used the apparatus shown in Figure 9.3 for the visualization of chromatograms by sublimation, but the principle is mentioned for the purpose of isolation by Ener (as reported by Mangold²³²).

CHAPTER 10

Quantitative Thin-Layer Chromatography

INTRODUCTION

Quantitative evaluation of thin-layer chromatograms can be divided into two general categories. In the first, the mixture components are assayed *on* the thin layer while in the second, they are eluted from the adsorbent before being measured. Either technique can be used with about 5 per cent error or, in ideal cases, about 3 per cent error.

The assay on the layer can be carried out by measuring the spot area, or the spot area and density on a developed and visualized chromatogram. These values are then related to the amount of substance in the spots through the use of standards or calibration curves. Spot size or density can also be measured on photocopies or photographs of visualized chromatograms and related to substance amounts. Finally, the assay can be carried out visually¹⁹⁴ by measuring the minimum visable amount of the standard on a chromatogram and making successive dilutions of an unknown solution until this minimum value is reached. The major advantages of these techniques are their simplicity and the fact that the chromatograms can be visualized with destructive spray reagents.

The elution of substances from the adsorbent introduces several sources of error which will be discussed. A spectrophotometric or colorimetric method must be available for the assay of the eluted compounds since the simple weighing of eluted samples gives poor re-

sults.⁴⁴² The elution technique has been used more extensively than the layer assay method.

ANALYSIS ON THIN LAYERS

Spot Area

This type of determination is based upon the fact that there is a mathematical relationship between the spot area for a given substance and the weight of the substance contained in the spot. The method involves only a small number of mechanical operations and is widely applicable.^{234,290,314,315,326,353,354}

There has, however, been some controversy over the type of mathematical relationship existing between these quantities. In fact, Stahl³⁷⁴ questioned the existence of any precise relation at all. Seher^{353,354} ignored the mathematics and obtained results with only 5 per cent error using calibration curves obtained by plotting spot area (from photostats) against weight. The calibration curves were not straight lines. Brenner and Niederwieser⁴⁴ and Pastuska and Petrowitz²⁹⁰ obtained a linear relationship by plotting the logarithm of the weight of sample against spot area. This agrees with Giddings and Keller,¹²³ who derived such a relationship for paper chromatography from theoretical considerations. The most thorough and successful study of this technique was made by Purdy and Truter,^{314,315} who found a linear relationship between the logarithm of the weight of a substance and the square root of the spot area. These authors noted, however, that a reasonably linear relationship resulted when the spot area or the logarithm of the spot area was used rather than its square root.

Purdy and Truter explored three general methods of analysis based upon spot size. Using quantities ranging from 1 to 100 γ , they carried out analyses for potassium and magnesium ions, hexadecanol, cholesterol, palmitic acid, cholestryl laurate and phenylazo-2-naphthol. Both adsorption and partition chromatography were involved. In all cases, it was necessary to have a pure sample of the substance in question for use as a standard.

The Graphical Method of Purdy and Truter. This method is simi-

lar to that of the Seher^{353,354} technique, that is, it is based upon the preparation of a calibration curve by plotting spot area against weight with no regard to linearity. A standard deviation of 4.3 per cent (25 observations) was obtained and the major error was ascribed to the difficulty of duplicating conditions on the calibration chromatograms and the analysis chromatograms. In contrast, the second and third methods are algebraic and depend upon the linear relationship between $\log W$ and \sqrt{A} .

First Algebraic Method of Purdy and Truter. In this method, a standard solution of the compound to be assayed is spotted on the layer with a solution of the unknown and a solution prepared by precise dilution of the unknown. The chromatogram is developed and visualized and the spot areas are determined. The results are calculated using Equation (1) where W and A are the weight and spot area of the unknown, W_s and A_s are the weight and area of the standard, A_d is the area of the diluted unknown spot and d is the dilution fac-

$$\log W = \log W_s + \left(\frac{\sqrt{A} - \sqrt{A_s}}{\sqrt{A_d} - \sqrt{A}} \right) \log d \quad (1)$$

tor. In essence, the unknown and its diluted sample establish the slope of the straight line used for the analysis and the standard establishes its intercepts. This method is the most accurate of the three, and yielded standard deviations of 2.7 per cent (540 observations) for adsorption chromatography and 3.6 per cent (982 observations) for partition chromatography.

Second Algebraic Method of Purdy and Truter. This method requires a pure sample of the substance to be assayed rather than a standard solution. The unknown solution is diluted in a precise manner and the diluted solution is divided into two portions. A known amount of the pure substance is added to one of the diluted samples and all three solutions are spotted on the same chromatogram. The chromatogram is developed and visualized; the spot areas are determined and the results are calculated using Equation (2). In this case,

$$\log \left(\frac{Wd + a}{W} \right) = \left(\frac{\sqrt{A_+} - \sqrt{A}}{\sqrt{A_d} - \sqrt{A}} \right) \log d \quad (2)$$

W and A are the weight and spot area of the unknown; A_d and A_+

are the spot areas of the diluted sample and the diluted sample containing added substance, respectively; a is the amount of pure substance added to the diluted unknown and d is the dilution factor. This method is somewhat less accurate and yielded standard deviations of 3.9 per cent (374 observations) for adsorption chromatography and 3.9 per cent (982 observations) for partition chromatography.

Purdy and Truter found that it was necessary to spot the same volume of each solution on a single chromatogram. They used an Agla micrometer syringe suspended 2 mm above the thin layer for this purpose. The appropriate sample was squeezed out of the syringe and transferred to the layer by raising the layer until it contacted the solvent drop on the syringe. Chloroform was found to be an unsatisfactory spotting solvent. The chromatograms were developed in the usual manner and those containing colorless compounds were visualized by spraying with conc. sulfuric acid and charring at 160° for 10 minutes. The areas were determined by laying a sheet of transparent paper on the chromatograms, tracing the outline of the spots and measuring the areas of the figures by superimposing the tracing on millimeter graph paper.

In a related method, Eble and Brooker⁹³ measured the length of the streaks (actually the distance between the origin and the zone front) formed when tryptamine was chromatographed on Kieselguhr G with acetone-water (99:1). The method gave results that were reproducible to about 5 per cent.

Spot Area and Density

The size and intensity of spots formed by the acid spray and charring of a chromatogram provide a satisfactory basis for quantitative evaluation.^{296,310,311} The method has an advantage in that the densities of the spots can be measured on a densitometer.

Additional variants are added, however, with respect to the spot-area methods discussed above. These are the carbon density of a given compound, its volatility and whether it chars in a uniform manner. These disadvantages can largely be nullified by the judicious use of internal standards and calibration curves⁸¹⁰ and results can be obtained with about 5 per cent error.

Before spotting, the thin layer, either the standard size of 5×20 cm^{310,311} or microscope slide size,²⁹⁶ is scanned in the densitometer to determine the degree and the uniformity of the background density. The chromatograms are then spotted, using a micropipette or micro-syringe, with the sample containing the substance to be assayed. The chromatogram is developed, sprayed with 50 per cent sulfuric acid or 25 per cent perchloric acid and charred in an oven. It is then scanned by passing it through a densitometer and the amount of unknown substance is calculated using an appropriate calibration curve. The densitometers which have been used are the Photovolt* models 52C and 521A with a stage attached for semiautomatic plotting of curves for the standard size layers^{310,311} and the Photovolt model 501A with transmission density unit no. 52 for the microchromatoplates.²⁹⁶ The slit sizes were 1×5 mm for standard size and 1×3 mm for microsize. Glycerides^{310,311} and cholesterol²⁹⁶ have been analyzed by this technique.

Spot Area and Density on Photostats or Photographs

The spot area has been determined from photostats of thin-layer chromatograms and used as a quantitative measurement by Kammerer (as reported in Mangold²³²) and by Seher.^{353,354} The error was about 5 per cent.

Neubauer and Mothes²⁷³ photographed chromatograms of opium alkaloid mixtures and cut the photographs into strips which were passed through a densitometer. Hefendehl¹⁴¹ made chromatograms transparent (see p. 104), photographed them and measured the density of the photographs against a calibration curve. He obtained results with about 5 per cent error in the analysis of menthofuran in an essential oil. Rybicka³³⁵ followed the glycerolysis of linseed oil by this method and was able to determine the optimum reaction conditions by finding the concentrations of the glycerides at timed intervals.

Minimum Spot Visibility

In this technique,¹⁹⁴ a standard solution of the substance to be assayed is successively diluted and each dilution is chromatographed un-

* Photovolt Corp., 95 Madison Avenue, New York, N.Y. A commercial densitometer, especially designed for thin-layer work, is now available from Photovolt.

til the minimum visible amount is determined. This will, of course, depend upon the visualization method and the eye of the analyst and will vary from one compound to another. The unknown solution is then successively diluted and chromatographed until the minimum visible amount is determined. Knowing the dilution factor of the unknown and the minimum value, the amount of unknown in the original sample can be calculated. The average error for nineteen samples was 11.3 per cent.

Using a similar method, Waldi has carried out analyses for pregnandiol in urine as an early test for pregnancy^{433,435} and for adrenaline (as its triacetyl derivative⁴³⁴) in adrenal extracts. He chromatographed the solution containing the material to be assayed on the same layer with a series of standard known concentrations and estimated the concentration of the unknown by comparison.

ANALYSIS AFTER ELUTION

Analysis by Spectrophotometry

Those compounds which possess a visible or ultraviolet spectrum are conveniently assayed in the following manner. After development of the chromatogram, the spot of adsorbent containing the substance to be analyzed is quantitatively removed; the substance is eluted with an appropriate solvent and assayed in a spectrophotometer. Thus, the spectroscopic properties of such compounds provide both a means of locating the spots on a developed chromatogram and a means of quantification. Substances which contain ultraviolet chromophores are more easily observed on phosphor-containing adsorbents (p. 21).

The methods involving elution contain several more sources of error than those in which compounds are assayed on the thin layers. First, the spots on the chromatogram must be located. This, however, is a minor problem when the substances have visible or ultraviolet chromophores. Second, the adsorbent containing the substance to be assayed must be completely removed from the glass support plate. Finally, the substance must be completely removed from the adsorbent before it is measured.

It has been suggested^{194,383,389} that the thin layers be prewashed

before being used for this type of analysis. This is carried out by allowing solvent to ascend (or descend) through the layers, perhaps in an apparatus similar to that shown in Chapter 3 (p. 49). The chromatogram is then spotted with a precise amount of substance to be separated and developed in a normal manner. The spots are located by viewing them in an appropriate light and the adsorbent containing the compound to be assayed is removed, normally with a razor blade. The compound is eluted from the adsorbent with a sufficiently polar solvent (one which will move the sample to the solvent front of a chromatogram) and measured in a spectrophotometer. The gadget described by Mottier²⁶⁹ (Figure 9.1) could well be used in this technique since it provides both a method for the removal of adsorbent from the glass plate and an elution tube, thus minimizing transfer losses. The method requires the preparation of a calibration curve and compounds which follow Beer's law. It is also necessary to make "blank" readings on the adsorbent, preferably on adsorbent which has been developed in a thin layer and is removed from a point on the layer comparable to the one containing the sample to be assayed.

Normally, the method gives results with an error of 3 to 5 per cent. It was first used for the assay of biphenyl in citrus fruits (2.8 per cent error^{194,380}) and has since been used for analysis of reserpine (about 5 per cent error³⁴⁴), coumestrol (5 per cent error²²²), menthyl salicylate,³⁸³ estrogens (standard deviation, 10 per cent³⁹⁴), alkaloids (5 per cent error⁴⁰⁰), bile acids (standard deviation, 3 per cent¹¹⁵), and *p*-hydroxybenzoic acid esters (3 per cent error¹¹⁸). Quinine alkaloids²⁷⁰ and 7-geranoxycoumarin⁴¹⁷ have been eluted and determined by fluorescence methods.

Analysis by Colorimetry

This technique is almost identical with the spectroscopic method described above except that a color-producing reagent is added to the sample before it is measured in the spectrophotometer. It is subject to the same disadvantages, although the location of the substances on the developed chromatogram is now a major problem. In general, the same nondestructive visualization techniques that are discussed in

Chapter 9 (p. 112) for preparative chromatography are applicable for the location of the spots to be eluted.

The method generally gives results with about 5 per cent error. It has been used for the analysis of esters (about 5 per cent error⁴²⁰), pregnanetriols (about 10 per cent error³⁹¹), citral (2 per cent error³⁸⁶), coumarin compounds³⁸⁸ and plasma phospholipids (about 5 per cent error¹³⁴). Further, the method has been successfully applied to the assay of carbohydrates,^{120,289} steroids,^{244,278,301,460} lipids^{413,430,458} and meprobamate.¹⁰²

RADIOACTIVITY METHODS

Analysis techniques which depend upon radioactivity require prior labeling of the compounds to be analyzed. The chromatograms are spotted and developed in the usual way and have been evaluated by three methods. The first method is to make autoradiographs^{231,234a} as described in Chapter 8 (p. 104) and to evaluate them using a densitometer and calibration curve. The second method^{234a} is to locate the spots by such an autoradiograph or by nondestructive spray reagents and to elute the sample and measure it in a conventional counter. Scintillator solutions have been used^{35,349,364} to aid the counting. Finally, Schulze and Wenzel³⁴⁰ used a gas-flow counter (Figure 7.1) for the assay of C¹⁴ and tritium labeled compounds with about 3 per cent error.

The application of labeling techniques to lipids has been thoroughly discussed by Mangold.^{232,234a}

CHAPTER 11

Specific Applications of Thin-Layer Chromatography

INTRODUCTION

This chapter will contain, in a tabular form, the majority of the specific applications of thin-layer chromatography which were recorded prior to January 1, 1963. Specific *Rf* values will not be given because of their notorious inconstancy on thin layers and because they are too numerous. It is hoped that this chapter will serve at least two purposes.

First, it will provide a general idea of the types of compounds which have been chromatographed on thin layers and, to some extent, the conditions of the separation. It would be hazardous, however, to assume that this compilation is complete because chromatographic conditions are usually incidental to a piece of research and are frequently buried in the experimental description.

Second, it will provide some idea of the adsorbents, solvents and visualization techniques which are commonly used in a given area or with a given class of compounds. In many cases, the same group of compounds has been treated in more than one fashion and the reader can choose the one which best suits his facilities and inclinations.

The numerous blank spaces in the tables are due to the failure of the original author to give specific details or to the present author's inability to obtain or translate the original article. In either case, they are given to make the compilation as complete as possible.

In many cases, a single reference will give details of several separate experiments such as the use of two adsorbents, the use of two techniques or the separation of two distinct groups of compounds. In other cases, two papers will report different aspects of the same experiments. This has been shown, as well as possible, by a system of partial lines. On occasion, a dotted line is used. This means that the regions on the ends of the dotted line are related and divided as shown but that the category crossed by the dotted line is the same for both experiments.

(EXPLANATION OF TABLES II.1 TO II.23)

Compounds

The various applications have, first of all, been arranged according to the class of compounds involved. This has been somewhat arbitrary at times and considerable overlapping could not be avoided. This is particularly serious between the sections devoted to amines, alkaloids and drugs and pharmaceuticals and between the essential oils and the terpenoids. In most cases, the applications are cross-referenced and the same example is reported in two places when it involves compounds falling in two classes. When the number of compounds in a specific entry is small, they are given by name. Otherwise, collective terms are used.

Within a given class, an attempt has been made to group the applications as much as possible according to the compounds involved. In most cases, the large comprehensive papers are given first and the others follow. The papers which came to the author's attention after the tables were first prepared, however, are given at the end of each section. The 2,4-DNPH's referred to in several cases mean the 2,4-dinitrophenylhydrazones.

Adsorbent

The adsorbent is given as a specific industrial product when it is reported as such in the original paper and when it is *especially prepared for thin-layer chromatography*. When a specific product is not named, it is implied that the original authors prepared their own ad-

sorbent and the reader is referred to Chapter 2. In most cases where partition chromatography is involved, the adsorbent is equilibrated with the developing solution or some of its components. This is generally noted.

The various materials which have been incorporated into the adsorbent for one reason or another are given in the tables, but without details. The reader is referred to Chapter 2.

Developer

The solvents used for development are given as such except when the article gives a very large number. In these cases, a selected few are given, and the number not listed is mentioned in the last item. Unless noted, the different solutions represent separate experiments and are not successive.

The proportions of the various solvent mixtures, unless noted, are volume-volume. The abbreviations listed on p. 87 have been used to conserve space. Otherwise, the names or, sometimes, the formulas (CCl_4 , CHCl_3 , H_2O , etc.) are used.

Visualization

For the sake of brevity, only the names of the spray or visualization reagents are given. The reader is referred to the alphabetical listing in Chapter 7 for exact details. Two abbreviations are used in this section; 2,4-DNPH for 2,4-dinitrophenylhydrazine and U.V. for ultraviolet visualization.

Comments

The author has tried to give the basic purpose of the chromatography in each case and has, on occasion, added notes about especially interesting applications.

TABLES 11.1-11.23 FOLLOW

TABLE II.I. ALDEHYDES, KETONES AND THEIR DERIVATIVES

| | Compound | Absorbent | Developer | Visualization | Comments | Ref. |
|---|---|--------------------------------------|--|---|--|------|
| 1 | Unsaturated aliphatic aldehydes and acyl- oins | Silica Gel G | a. pet. ether-EtOEt (90:10) (95:5) b. pet. ether-EtOEt-HOAc (70:30:1) | I ₂ vapor | a. diagnostic b. monitored a syn- thesis | 119 |
| 2 | Long-chain (over eight carbons) aliphatic ketones | Silica Gel G | a. benzene-EtOEt mixtures b. toluene-EtOEt mixtures c. pet. ether-EtOEt mixtures | phosphomolybdic acid | diagnostic | 238 |
| 3 | Biologically active aldehydes | | | | | 447 |
| 4 | β -Dicarbonyl com- pounds | starch-bound silica gel | benzene-EtOAc (7:3) | | diagnostic | 14 |
| 5 | Cyclohexanodiones | see "Miscellaneous" No. 12 | | | | 254 |
| 6 | Aliphatic and aromatic aldehydes and ketones | unbound aluminum os- ide | a. benzene b. benzene-EtOH (98:2)(95:5) (90:10) c. CHCl ₃ d. EtOEt e. pet. ether-benzene (1:1) | reagents used for paper chromatography | diagnostic | 144 |
| 7 | Aromatic aldehydes and ketones | plaster-of-Paris-bound silica gel | hexane-EtOAc (4:1)(3:2) | | diagnostic | 113 |
| 8 | Phenolic aldehydes | see "Phenols, etc." No. 12 | | | | 290 |
| 9 | Vanillin and other substituted benzal- dehydes | Silica Gel G | a. pet. ether-EtOAc (2:1) b. hexane-EtOAc (5:2) c. CHCl ₃ -EtOAc (98:2) d. decalin-CH ₂ Cl ₂ -MeOH (5:4:1) | a. hydrazine sulfate in HCl b. methanolic KOH c. methanolic KOH fol- lowed by diazotized sulfanilic acid | diagnostic | 396 |

| | | | | | | |
|----|--|--|--------------------------------|--|--|-----|
| 10 | Cholestane-dione-1,3 | see "Steroids" No. 45 | | | | |
| 11 | Aldehydes and ketones in peppermint oil | see "Essential Oils" No. 6 | | | | |
| 12 | Acetals and their determination in thin layers | | | | | 206 |
| 13 | 2,4-Dinitrophenyl-hydrazines of aldehydes | Silica Gel G a. benzene-pet. ether (3:1) for aliphatic b. benzene-EtOAc (95:5) for aromatic | colored | | | 84 |
| 14 | 2,4-DNPH's of ketones | starch-bound SiO ₂ -Bentonite (4:1) a. benzene b. benzene-pet. ether-EtOEt mixtures | colored | | | 209 |
| 15 | 2,4-DNPH's of aliphatic aldehydes and ketones | Silica Gel G benzene-EtOAc (95:5) | a. colored b. alcoholic KOH | | | 258 |
| 16 | 2,4-DNPH's of aliphatic aldehydes | polyvinyl alcohol-bound silica gel benzene-H ₂ O (91:8:8:2) | colored | | | 284 |
| 17 | 2,4-DNPH's of aldehydes and ketones | unbound aluminum oxide a. benzene-hexane (1:1) b. EtOEt | colored | | | 331 |
| 18 | 2,4-DNPH's of aliphatic aldehydes | starch-bound ZnCO ₃ small amounts of pyridine in pet. ether-benzene mixtures | colored | | | 9 |
| 19 | 2,4-DNPH's of hydroxy aldehydes and ketones | a. Aluminum Oxide G b. Silica Gel G toluene-EtOAc (3:1)(1:1) | colored | | | 5 |

Thin-Layer Chromatography

TABLE 11.2. ALKALOIDS

| | Compound | Adsorbent | Developer | Visualization | Comments | Ref. |
|---|--|--|---|--|--|------|
| 1 | Fifty-four misc. alkaloids | Silica Gel G | a. $\text{CHCl}_3\text{-acetone-diethylamine}$ (3:4:1) b. $\text{CHCl}_3\text{-diethylamine}$ (9:1) c. cyclohexane- $\text{CHCl}_3\text{-diethylamine}$ (5:4:1) d. cyclohexane-diethylamine (9:1) e. benzene-EtOAc-diethylamine (7:2:1) | potassium iodoplatinate | a. diagnostic b. devised an identification system | 436 |
| | | Aluminum Oxide G | f. CHCl_3 g. cyclohexane- CHCl_3 (3:7) plus 0.05% diethylamine | | | |
| | | Silica Gel G-NaOH | h. MeOH | paper chromatography reagents | diagnostic | 144 |
| 2 | Thirty-seven misc. alkaloids | unbound aluminum oxide | a. benzene-EtOH mixtures b. CHCl_3 c. EtOH | paper chromatography reagents | diagnostic | |
| 3 | Twenty-one indole derivatives | Silica Gel G | a. MeOAc-2-propanol-NH ₃ OH (Ca, 25% NH ₃) (45:35:20) b. $\text{CHCl}_3\text{-HOAc}$ (95:5) | a. Van Urk reagent b. Prochasko reagent c. 2,4-dinitrophenylhydrazine | a. diagnostic b. two dimension | 376 |
| 4 | <i>Amaryllidaceae</i> , <i>Papaveraceae</i> and Bisbenzylisoquinoline alkaloids (total 57) | Silica Gel G Silica Gel G-NaOH | EtOAc- $\text{CHCl}_3\text{-MeOH}$ (20:20:10) | Dragendorff's reagent | diagnostic | 83 |
| 5 | Alkaloid-containing drug mixtures | Silica Gel G with fluorescent material | a. MeOH-HOAc-EtOEt-benzene (1:18:60; 120) b. EtOH-HOAc-benzene (12:5:80) c. EtOEt- $\text{CHCl}_3\text{-MeOH}$ (50:50:1) d. benzene-EtOH (9:1) e. BuOH-HOAc (10:1) | a. $\text{FeCl}_3\text{-K}_3\text{Fe}(\text{CN})_6$ b. chloramine T-HCl-NH ₃ c. potassium iodoplatinate d. 2,6-dichloroquinone chlorimide e. ninhydrin f. SbCl_5 in CCl_4 g. CNBr and benzidine | diagnostic | 117 |

| | | | | | | |
|----|---|--|---|--|--|------------|
| 6 | Alkaloids and barbiturates in toxicology | Silica Gel G | a. MeOH b. CHCl ₃ -Et ₂ OEt (85:15) | a. Dragendorff's reagent b. I ₂ and KI in HOAc c. sodium fluorescein d. FeCl ₃ in HOAc e. Zwicky's reagent f. KMnO ₄ in HOAc | a. diagnostic b. clinical | 226 227 |
| 7 | Sixteen alkaloids and nitrogen containing drugs | Silica Gel G | a. EtOH-pyridine-dioxane-H ₂ O (50:20:25:5) b. EtOH-HOAc-H ₂ O (60:30:10) c. EtOH-dioxane-benzene-NH ₄ OH (5:40:50:5) d. MeOH-BuOH-benzene-H ₂ O (60:15:10:15) Aluminum Oxide G | potassium iodoplatinate | a. diagnostic b. urine assay | 57 |
| 8 | Opium alkaloids | alumina buffered to pH 5 with acetate | H ₂ O-EtOH-BuOH (1:1:9) | U.V. | diagnostic | 239 |
| 9 | Opium alkaloids | Silica Gel G | benzene-MeOH (8:2) | Dragendorff's reagent | a. diagnostic b. quantitative c. preparative | 273 |
| 10 | Ergot alkaloids | a. cellulose powder impregnated with formamide b. Silica Gel G | a. benzene-heptane-CHCl ₃ (6:5:3) | a. Dragendorff's reagent b. With formamide layers the excess formamide was first destroyed by spraying with 0.25% NaNO ₂ in 0.5% HCl and Dragendorff's reagent was used. c. U.V. d. K ₃ Fe(CN) ₆ | a. diagnostic b. quantitative | 399 400 |
| | Morphine alkaloids | c. Silica Gel G-NaOH d. Cellulose powder impregnated with formamide | b. CHCl ₃ -EtOH (9:1) c. benzene-heptane-CHCl ₃ -diethylamine (6:5:3:0.03) | | | |
| 11 | Alkaloids of <i>Athaea</i> species | Silica Gel G | CHCl ₃ -MeOH (1:2) | a. Potassium iodoplatinate b. H ₂ SO ₄ | diagnostic | 285 |

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TABLE 11.2—Cont.

| | Compound | Absorbent | Developer | Visualization | Comments | Ref. |
|----|--|------------------------------------|---|---|--|------|
| 12 | Lysergic acid alkaloids | | | | diagnostic | 151 |
| 13 | <i>Aspidosperma</i> alkaloids | Silica Gel G (not activated) | Methyl cellosolve | U.V. | diagnostic | 215 |
| 14 | Quinine alkaloids | Silica Gel G (prepared in acetone) | "Shellisol" (23:9:9) | T-diethylamine-acetone exposed to formic acid vapor and U.V. | a. diagnostic b. quantitative | 270 |
| 15 | An isomer of rhoecadin from <i>Papaver rheas</i> | Aluminum Oxide G | a. benzene-CHCl ₃ (1:2) b. benzene-tetrahydrofuran (95:5) c. CHCl ₃ -MeOH (9:1) | Dragendorff's reagent | diagnostic | 444 |
| 16 | Morphine | Silica Gel G | BuOH-HOAc (9:1) saturated with H ₂ O | <i>p</i> -dimethylaminobenzaldehyde in H ₂ SO ₄ | a. diagnostic b. on microscopic slides | 437 |
| 17 | Tropine and <i>pseudo</i> tropine | Silica Gel G | BuOH-NH ₄ OH (28% NH ₃) (8:2) | Dragendorff's reagent | diagnostic | 188 |
| 18 | Anafrine (a piperidine alkaloid) | Silica Gel G | a. MeOH-CHCl ₃ -diethylamine (1:9:0.5) b. EtOH-NH ₄ OH (conc.) (8:2) | Dragendorff's reagent | diagnostic | 332 |
| 19 | Reserpine | Silica Gel G | MeOH-methyl ethyl ketone-heptane (8:4:33, 6:58) | U.V. | a. diagnostic b. quantitative c. developed in dark | 344 |

TABLE 11.3. AMINES AND THEIR DERIVATIVES

| | Compound | Adsorbent | Developer | Visualization | Comments | Ref. |
|---|---|-----------------------------------|---|--|--|------------|
| 1 | Simple aliphatic amines | Silica Gel G | a. EtOH (95%)·NH ₄ OH (25% NH ₃) b. phenol-H ₂ O (8:3) c. butanol-HOAc-H ₂ O (4:1:5) | ninhydrin | diagnostic | 399 |
| | | Silica Gel G buffered to pH 6.8 | d. EtOH-H ₂ O (70:30) | | | |
| 2 | Strong-base amines | unbound aluminum oxide | a. acetone-heptane (1:1) b. CHCl ₃ /NH ₃ (sat. at 22°)-EtOH (96%) (30:1) c. acetone-MeOH-H ₂ O (8:2:1) d. methyl ethyl ketone-H ₂ O (15:1) | I ₂ vapor-U.V. | a. diagnostic b. describes descending development with unbound layers | 235 256 |
| 3 | Diphenylamine derivatives | Silica Gel G | a. CHCl ₃ b. toluene c. benzene | NaNO ₂ and H ₂ SO ₄ | a. diagnostic b. analysis of powder | 138 |
| 4 | Tryptamine | Kieselguhr G | acetone-H ₂ O (99:1) | FeCl ₃ -K ₄ Fe(CN) ₆ | quantitative | 93 |
| 5 | Aromatic amines and nitro compounds | Plaster-of-Paris-bound silica gel | | a. KOH in acetone b. NaCl in conc. HCl c. HNO ₃ -H ₂ SO ₄ | diagnostic | 110 |
| 6 | Nitramine explosives | see "Miscellaneous", No. 10 | | | | 216 |
| 7 | Piperidoles and quinolols | see "Miscellaneous", No. 12 | | | | 254 |
| 8 | Twelve pteridines | Silica Gel G | a. dimethylformamide-H ₂ O (190:10) (20:1:80) b. 5% citric acid c. NH ₄ OH (25% NH ₃) d. twenty others | U.V. | diagnostic | 274 |
| 9 | Simple amines and diamines by electrophoresis | Silica Gel G buffered with borax | electrolyte: EtOH-H ₂ O (30 ml. plus 30 ml.) with 2 g. NaOAc | ninhydrin | diagnostic | 291 |

TABLE 11.4. CARBOHYDRATES, GLYCOSIDES AND THEIR DERIVATIVES

| | Compounds | Adsorbent | Developer | Visualization | Comments | *Ref. |
|---|-----------------------|---|---|--|--|-------|
| 1 | Thirteen misc. sugars | Kieselguhr G buffered with NaOAc | EtOAc-2-propanol-H ₂ O (65:23:4:11:6) | anisaldehyde and H ₂ SO ₄ | diagnostic | 377 |
| 2 | Simple sugars | Polyacrylomethyl-Kieselguhr G-Aluminum Oxide G (5:6:1) buffered with NaHPO ₄ | n-amyl alcohol-2-propanol-HOAc-H ₂ O (55:15:110:30) | aniline phthalate | diagnostic | 36 |
| 3 | Simple sugars | Silica Gel G buffered with boric acid | a. benzene-HOAc-MeOH (1:1:3) b. methyl ethyl ketone-HOAc-MeOH (3:1:1) | naphthoresorcinol and H ₂ SO ₄ | a. diagnostic b. quantitative | 289 |
| 4 | Simple sugars | unbound Cellulose Powder MN-300 | a. EtOAc-pyridine-H ₂ O (2:1:2) b. phenol, equilibrated with 1% NH ₄ OH c. 2-propanol-pyridine-HOAc-H ₂ O (80:80:10:4) | anisidine phthalate | diagnostic | 350 |
| 5 | Simple sugars | Silica Gel G | n-PrOH-conc. NH ₄ OH-H ₂ O (6:2:1) | a. ninhydrin b. aniline hydrogen phthalate | a. diagnostic b. preparative c. noted sugar amination during development | 439 |
| 6 | Simple sugars | a. Silica Gel G b. Silica Gel G buffered with boric acid | a. PrOH-EtOAc-H ₂ O (3:2:1) b. BuOH-acetone-H ₂ O (4:5:1) c. PrOH-EtOAc-H ₂ O (7:2:1) d. methyl ethyl ketone-HOAc-H ₂ O (3:1:1) e. Sol. b with 5% NH ₄ OH f. EtOAc-2-PrOH-H ₂ O (65:24:12) g. Kieselguhr G buffered with 0.02 N NaOAc | naphthoresorcinol and H ₂ PO ₄ | a. diagnostic b. used wedge-shaped chromatograms | 307 |

| | | | | | | |
|----|--|--|---|---|--|-----|
| 7 | Glucose and trehalose | Silica Gel G | <i>sec</i> -BuOH saturated with H ₂ O | NaIO ₄ -KMnO ₄ | a. diagnostic b. could not separate more complex mixtures | 454 |
| 8 | Ten sugar acetates and eight inositol acetates | Silica Gel G | benzene with 2-10% MeOH | a. H ₂ O b. ferric hydroxamate reagent | a. diagnostic b. preparative c. multiple development | 398 |
| 9 | Sugar acetates | starch-bound acid | silicic a. EtOAc-benzene (3:7) b. MeOH-benzene (2:98) | silver nitrate-NH ₃ -NaOMe | diagnostic | 75 |
| | Sugar benzoates | | c. EtOAc-benzene (3:97)(4:6) | naphthoresorcinol-H ₃ PO ₄ | diagnostic | 308 |
| 10 | Partially methylated sugars, isopropylidene sugars and other derivatives | a. Silica Gel G b. Silica Gel G with boric acid | a. BuOH-acetone-H ₂ O (4:5:1) b. methyl ethyl ketone-HOAc-H ₂ O (3:1:1) c. pet. ether | naphthoresorcinol-H ₃ PO ₄ | a. diagnostic b. quantitative | 120 |
| 11 | Sucrose and raffinose | Starch-bound silica gel | a. 2-PtOH-toluene-EtOH-H ₂ O (10:2:5:2.5) b. toluene-EtOAc-EtOH (95%) (10:5:5) | naphthoresorcinol-H ₃ PO ₄ 2'-7'-dichlorofluorescein | a. diagnostic b. quantitative | 441 |
| | Sucrose esters | | | anisaldoxyde-H ₂ SO ₄ -HOAc | diagnostic | 5 |
| 12 | Maltodoligosaccharides up to ten glucose units | Kieselguhr G | a. BuOH-pyridine-H ₂ O (65:20:15) and six other combinations b. BuOH-EtOH-H ₂ O (50:30:20) c. BuOH-2,6-lutidine-H ₂ O (60:30:10) | | | 203 |
| 13 | Aldose 2,4-DNPH's | a. Aluminum Oxide G b. Silica Gel G | a. toluene-EtOAc (1:1) b. toluene-EtOAc (3:1) (1:1) | a. colored compounds b. NaOH | diagnostic | 7 |
| 14 | Sugar derivatives | alumina | | | | |
| 15 | <i>p</i> -Bromophenoxylosazones of xylose derivatives | plaster-of-Paris-bound silica gel | benzene-MeOH (9:1) | colored compounds | | |

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TABLE 11.4—Cont.

| Compound | Absorbent | Developer | Visualization | Comments | Ref. |
|---|--|---|--|-----------------------------|------|
| 16 Cardiac glycosides | unbound, deactivated (H ₂ O or HOAc) silica gel | benzene-EtOH (3:1) saturated with H ₂ O | a. trichloroacetic acid-chloramine-T b. SbCl ₃ and acetic anhydride in CHCl ₃ | diagnostic | 322 |
| 17 Cardiac glycosides | Silica Gel G | EtOAc-pyridine-H ₂ O (5:1:4), upper phase | SbCl ₃ in CHCl ₃ | diagnostic | 392 |
| 18 Cardiac glycosides | Silica Gel G | a. CHCl ₃ -MeOH (9:1) b. CHCl ₃ -acetone (65:35) | H ₂ SO ₄ and acetic anhydride | diagnostic | 378 |
| 19 Glycosides of <i>atracytis gummifera</i> | Silica Gel G | a. BuOH-MeOH-H ₂ O (8:1:1) b. PrOH-xylene-H ₂ O (7:2:1) | conc. H ₂ SO ₄ | diagnostic | 100 |
| 20 k-Strophanthin | Silica Gel G | BuOH-MeOH-formamide (17:2:1) | a. SbCl ₃ in CHCl ₃ b. trichloroacetic acid-chloramine-T c. Kedde reagent d. conc. H ₂ SO ₄ | diagnostic | 223 |
| 21 Flavone and coumarin glycosides | Silica Gel G | EtOAc-methyl ethyl ketone-formic acid-H ₂ O (5:3:1:1) | diphenylboric acid, β -aminoethyl ester | diagnostic | 379 |
| 22 Flavone glycosides | starch-bound silica gel | a. EtOAc-CHCl ₃ (95:5) b. EtOAc-EtOH (95:5) c. 3-methyl-1-butanol-hexane-HOAc-H ₂ O (3:1:3:3) | a. AlCl ₃ b. uronium acetate | diagnostic | 286 |
| 23 Flavanol glycosides | Silica Gel G | a. HOAc-formic acid-H ₂ O-MeOH (10:2:2:1) | NH ₃ vapor | diagnostic and quantitative | 126 |
| Cardiac glycosides | — | b. methyl ethyl ketone-volucene-H ₂ O-HOAc-MeOH (40:5:3:1:2:5) | SbCl ₃ in CHCl ₃ | — | — |

| | | | | | | |
|----|-------------------------------------|--|--|--|--|-----|
| 24 | Rutin | a. unbound polyamide powder | a. MeOH b. MeOH-H ₂ O (8:2) (6:4) | a. diazotized sulfanilic acid b. U.V. | diagnostic | 74 |
| | | b. Silica Gel G | c. BuOH-HOAc (9:1) saturated with H ₂ O | <i>p</i> -dimethylaminobenzaldehyde in H ₂ SO ₄ | a. diagnostic b. on microscope slides | 437 |
| 25 | Brain gangliosidic sub- stances | Silica Gel G | PrOH-1 N NH ₄ OH-H ₂ O (6:2:1) | | a. diagnostic b. monitored column | 440 |
| 26 | Catalpa glucosides | Silica Gel G | a. MeOH b. MeOH-EtOEt mixtures | conc. H ₂ SO ₄ | diagnostic | 39 |
| 27 | Anthocyanins and their aglucones | polyacrylonitrile-“Per- lon” (7:1) buffered with K ₂ HPO ₄ | 1-pentanol-1-propanol-HOAc-H ₂ O (3:2:2:1) plus 2 parts 1-heptanol | | diagnostic | 36 |
| 28 | Misc. glycosides | | | | | 189 |

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TABLE 11.5. CARBOXYLIC ACIDS AND THEIR DERIVATIVES (EXCLUSIVE OF FATTY ACIDS)

| | Compound | Absorbent | Developer | Visualization | Comments | Ref. |
|---|---|---|---|--|----------------------------------|-------------------|
| 1 | 2,6-Dimethyl-2,6-octadienoic acid (Hildebrandt's acid) | Silica Gel G | CHCl ₃ :HOAc (97:3) | KMnO ₄ | diagnostic | 428 |
| 2 | Dicarboxylic acids | Silica Gel G | a. benzene-MeOH-HOAc (45:8:4) b. benzene-dioxane-HOAc (90:25:4) | bromphenol blue acidified with citric acid | diagnostic | 302 |
| 3 | Carboxylic acids (as ammonium salts) | Silica Gel G | a. EtOH(95%)-H ₂ O-NH ₄ OH(25% NH ₃)(100:12:16) | brom cresol green | diagnostic | 42 |
| 4 | Methoxy-piperonylic acids | Silica Gel G | EtOAc-hexane-HOAc (50:50:0.5) | chromotropic acid-H ₂ SO ₄ | diagnostic | 32 |
| 5 | Phenolic acids | see "Phenols, Alcohols, etc.", No. 9 and No. 12 | | | | 225 288 290 |
| 6 | Aromatic acids | a. gelatin-bound Silica Gel G "Perlon" (0.5:7:4) b. polyacryloylurethane "Perlon" (8:4) | a. <i>n</i> -amyl alcohol- <i>n</i> -BuOH-EtOAc-HOAc-H ₂ O (60:20:30:10:5) b. <i>n</i> -octane-EtOAc- <i>n</i> -amyl alcohol-EtOEt-formic acid (40:50:15:15:10) | U.V. pentane-HOAc (88:12) | diagnostic | 36 |
| 7 | <i>p</i> -Hydroxybenzoic acid esters | Silica Gel G with fluorescent material | | U.V. | quantitative | 118 |
| 8 | Phthalates, phosphates and other esters as plasticizers | see "Miscellaneous," No. 14 | | | | 293 |
| 9 | Gallic acid and its esters | unbound powder | polyamide a. MeOH b. EtOH c. EtOEt d. CCl ₄ -MeOH (7:3) e. six others | | a. diagnostic b. antioxidants | 69 |

| | | | | | |
|----|---|---|---|---|------------|
| 10 | Lactones, lactams and thiolactones | Silica Gel G | a. isopropyl ether b. isopropyl ether-EtOAc (80:20) c. isopropyl ether- <i>iso</i> -octane (20:80) (60:40) | a. NaOH-hydroxylamine-HOAc-FeCl ₃ consecutively b. Dragendorff's reagent | 207 |
| 11 | Dicarboxylic acids (from oxalic to sebacic) and carbocyclic acids | Kieselguhr G-Polyethylene glycol (M-1000) (2:1) | isopropyl ether-formic acid-H ₂ O (90:7:3) | bromcresol purple | 200 201 |

Thin-Layer Chromatography

TABLE 11.6. DRUGS AND PHARMACEUTICALS

| | Compound | Adsorbent | Developer | Visualization | Comments | Ref. |
|----|---|--|--|---|--------------------------------------|------------|
| 1 | Caffeine, theobromine and theophylline | Silica Gel G | a. EtOAc-MeOH-12 N HCl (18:2:0.05) b. EtOAc-MeOH-HOAc (8:1:1) c. CHCl ₃ -MeOH (19:1) | sublimation from layer | a. diagnostic b. preparative | 10 |
| 2 | Meprobamate | starch-bound silicic acid | EtOH-cyclohexane (15:85) | conc. H ₂ SO ₄ | a. diagnostic b. quantitative | 102 240 |
| 3 | Barbiturates | see "Alkaloids," No. 6 | | | | 226 227 |
| 4 | Nitrogen-containing drugs | Silica Gel G | a. organic b. buffer solutions depending upon drug solutions | a. I ₂ in CHCl ₃ b. potassium iodoplantate | a. diagnostic b. semiquantitative | 279 |
| 5 | Narcotics in forensic chemistry | see "Miscellaneous," No. 19 | | | | 92 |
| 6 | Alkaloidal drugs in urine | see "Alkaloids," No. 7 | | | | 57 |
| 7 | Urinary metabolites of psychotropic drugs | Silica Gel G | BuOH-HOAc-H ₂ O (88:5:7) (65:15:20) | conc. H ₂ SO ₄ | diagnostic | 101 |
| 8 | Chloramphenicol | aluminum oxide | | | monitored synthesis | 217 |
| 9 | Penicillin preparations | Silica Gel G | a. acetone-MeOH (1:1) b. 2-PrOH-MeOH (30:70) | sodium azide-I ₂ solution | diagnostic | 103 |
| 10 | Tetracyclines, penicillins and rifomycins | a. Silica Gel G b. Silica Gel G buffered to pH 4.5 or 4.2 | a. aqueous citric acid solutions b. pure alcohols c. many others | a. HCl and others b. microbiological assay | diagnostic | 275 276 |
| 11 | Antibiotics (macrolides) | Silica Gel G | acetone-H ₂ O (98:2) | microbiological assay | diagnostic | 33 |

| | | | | | | |
|----|-----------------------------------|---|---|--|--|------------|
| 12 | Sulfonamides | a. Silica Gel G b. Silica Gel G containing fluorescein | CHCl ₃ -EtOH-heptane (1:1:1) plus 1.0 to 1.8% H ₂ O | a. HCl-NaNO ₂ -N(1-naphthyl) ethylenediamine b. U.V. | diagnostic | 196 |
| 13 | Sulfonamides | Silica Gel G | CHCl ₃ -EtOH-heptane (1:1:1) | <i>p</i> -dimethylaminobenzaldehyde, acidified | diagnostic | 452 |
| 14 | Sulfonamide, radioactive | Silica Gel G | BuOH sat. with conc. NH ₃ | radioactive method | a. diagnostic b. quantitative c. preparative | 349 |
| 15 | Misc. constituents of plant drugs | | | | | 379 |
| 16 | Pharmaceutical applications | | | | review | 122 340 |

Thin-Layer Chromatography

TABLE 11.7. DYES

| | Compound | Adsorbent | Developer | Visualization | Comments | Ref. |
|---|--|--------------------------------------|---|--|---|-------------------|
| 1 | Food dyes | unbound aluminum oxide | a. $\text{Ba(OH}\cdot\text{EtOH}\cdot\text{H}_2\text{O}$ (90:10:10) (80:20:10)(70:30:30) (60:40:40)(50:50:50) | colored compounds | a. diagnostic b. spot made alkaline before chromatography c. circular | 266 269 306 |
| 2 | Food dyes | Cellulose Powder MN 300 G and MN 300 | a. aqueous sodium citrate (2.5%) NH_4OH (25%) (4:1) b. $\text{P(OH-EtOAc-H}_2\text{O}$ (6:1:3) c. $t\text{-BuOH}$ -propanoic acid- H_2O (50:12:38) with 0.4% KCl | colored compounds | diagnostic | 451 |
| 3 | Food dyes | unbound aluminum oxide | a. MeOH b. EtOH c. CHCl_3 d. CCl_4 e. EtOEt f. seven others | colored compounds | a. diagnostic b. quantitative | 73 |
| 4 | Food dyes | Silica Gel G | a. CHCl_3 -acetic anhydride (75:2) b. benzene c. methyl ethyl ketone- HOAc-MeOH (40:5:5) | SiO_2Cl in CHCl_3 | diagnostic | 257 |
| 5 | Carotenoid dyes | see "Vitamins," No. 2 | | | | |
| 6 | Carotenoid dyes, synthetic | calcium hydroxide-Silica Gel G (6:1) | pet. ether-benzene (2:3) | colored compounds | a. diagnostic b. monitored synthesis | 166 |
| 7 | Dyes in gasoline | Silica Gel G | benzene | colored compounds | a. diagnostic b. to catch gasoline thieves | 139 |
| 8 | Test mixture dyes: butter yellow, Sudan red III and indophenol | see "Miscellaneous," No. 16 | | | | 381 |

TABLE II.3. ESSENTIAL OILS

| | Compound | Absorbent | Developer | Visualization | Comments | Ref. |
|---|------------------------------|---|---|--|--|-------------------|
| 1 | Essential oils of Argentina | starch-bound silicic acid containing fluorescent material | a. hexane b. pet. ether-Et ₂ O-Ac mixtures | a. U.V. b. fluorescein-Br ₂ c. bromoresol green d. 2,4-DNPH e. conc. H ₂ SO ₄ | diagnostic | 105 106 107 |
| 2 | Thirteen essences | starch-bound silicic acid | a. hexane b. benzene-ethanol mixtures | a. SbCl ₃ in CHCl ₃ b. 2,4-DNPH c. KMnO ₄ d. <i>p</i> -dimethylaminobenzaldehyde-H ₂ SO ₄ | diagnostic | 287 |
| 3 | Misc. essential oils | Silica Gel G | benzene-CHCl ₃ (1:1) | SbCl ₃ in CHCl ₃ | a. diagnostic b. used with gas chromatography | 382 |
| 4 | Perfume industry | Silica Gel G | a. hexane b. benzene c. CH ₂ Cl ₂ d. CHCl ₃ | a. SbCl ₃ in CHCl ₃ b. SbCl ₅ in CCl ₄ c. conc. H ₂ SO ₄ d. vanillin and other aldehydes in H ₂ SO ₄ e. fluorescein-Br ₂ f. 2,4-DNPH g. <i>o</i> -dianisidine | a. diagnostic b. circular c. in shaped areas | 368 |
| | a. Essential oils | — | — | — | — | |
| | b. Single essences | — | — | — | — | |
| | c. Balsams and resins | — | — | — | — | |
| | d. Tar and tar products | — | — | — | — | |
| | e. Phenols and phenol ethers | — | — | — | — | |
| | f. Wool wax and derivatives | — | — | g. CHCl ₃ -MeOH mixtures | h. SbCl ₅ in CCl ₄ i. acetic anhydride-H ₂ SO ₄ | |
| | g. Azulenes | — | — | h. hexane i. benzene | j. chloranil in CHCl ₃ k. Br ₂ in CHCl ₃ | |
| 5 | Misc. essential oils | see "Terpenoids," No. 1 | — | — | — | 190 |

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TABLE 11.8—Cont.

| | Compound | Adsorbent | Developer | Visualization | Comments | Ref. |
|----|--------------------------------|---|---|---|---|------------|
| 6 | a. Misc. essential oils | starch-bound silicic acid containing fluorescent material (Rhodamine 6 G) | a. hexane b. hexane-EtOAc mixtures | a. U.V. b. 2,4-DNPH | diagnostic | 323 |
| | b. Oils of <i>Mentha</i> genus | — | — | — | — | 324 |
| | c. Mint oils | — | — | — | quantitative | 326,327 |
| 7 | Oils of Japanese mint | starch-bound silicic acid | hexane-EtOAc (85:15) | vanillin in H ₂ SO ₄ | diagnostic | 325 |
| 8 | Oils of <i>Mentha piperita</i> | Silica Gel G | hexane | a. SbCl ₃ in CrCl ₃ b. transparency method | quant. assay of menthofuran | 167 168 |
| 9 | Oils of peppermint | — | — | — | — | 297 |
| 10 | Oils of <i>Oleum menthae</i> | Silica Gel G | a. benzene-EtOAc (95:5) b. hexane | a. vanillin in H ₂ SO ₄ b. SbCl ₃ in CH ₂ Cl ₂ c. SbCl ₅ in CCl ₄ d. fluorescein-Br ₂ e. 2,4-DNPH f. KI in HOAc with Na ₂ SO ₃ and followed by starch g. HNO ₃ -HOAc (1:300) | diagnostic | 171 |
| 11 | Oils of peppermint | plaster-of-Paris-bound silicic acid | “Skellysolve” B-EtOAc mixtures, up to 10% EtOAc | a. transmitted light b. U.V. c. trichloroacetic acid in CHCl ₃ d. KMnO ₄ in H ₂ SO ₄ e. Rhodamine B | a. diagnostic b. study site and mode of terpene formation in plant c. preparative | 19 20 |
| 12 | Menthol in peppermint oil | alumina-silicate | — | — | quantitative | 133 |

| | | | | | | |
|----|---|--|---|---|--|-----|
| 13 | Oils of Eucalyptus | starch-bound silica gel | hexane-EtOAc (85:15) | conc. H ₂ SO ₄ | a. diagnostic b. circular | 313 |
| 14 | Oils of Eucalyptus | starch-bound magnesol | | | a. diagnostic b. circular | 51 |
| 15 | Oils of <i>Strobilanthes</i> <i>Limifolia</i> | a. silica gel ("Celite")-starch (16:4:1) b. plaster-of-Paris-bound silica gel | hexane-EtOAc mixtures | | a. diagnostic b. monitored column | 58 |
| 16 | Oil of jasmine | see "Terpenoids," No. 13 | | | | |
| 17 | Azulene of camphor blue oil | see "Terpenoids," No. 14 | | | | |
| 18 | Oil of <i>Pogostemon patchouli</i> Pell | starch-bound silicic acid | a. hexane-EtOAc (85:15) b. solution a plus 5% CHCl ₃ | conc. H ₂ SO ₄ | a. diagnostic b. used "chromato-bars" | 108 |
| 19 | Oils of <i>Aniba montana</i> L. | starch-bound silicic acid and <i>terra siccata</i> | CHCl ₃ | conc. H ₂ SO ₄ | diagnostic | 124 |
| 20 | Oils of <i>grapefruit</i> | a. starch-bound silicic acid b. plaster-of-Paris-bound silicic acid | a. hexane b. 2,2-dimethylbutane c. cyclohexane d. methylcyclohexane e. isopentane | a. fluorescein-Br ₂ b. HNO ₃ -H ₂ SO ₄ (5:95) | a. diagnostic b. predicted solvents for column work | 131 |
| b. | Oxygenated compounds | — | — | f. hexane-EtOAc mixtures g. EtOAc-CHCl ₃ (alcohol free) mixtures h. EtOAc-benzene (15:85) i. hexane-nitropropane j. isopropyl ether-hexane (50:50) | | 191 |
| 21 | Oils of orange juice | starch-bound silicic acid | | hexane-EtOAc (83:15) plus a variety of others | diagnostic | 192 |
| | | | | a. fluorescein-Br ₂ b. 2,4-DNPH c. o-dianisidine d. conc. sulfuric-nitric acid | | |

Thin-Layer Chromatography

TABLE 11.8—*Cont.*

| Compound | Absorbent | Developer | Visualization | Comments | Ref. |
|--|--|--|---|---|-------------------|
| 22 Oils of lemon a. Menthyl salicylates | starch-bound silicic acid with phosphors | a. EtOEt-pet. ether mixtures | a. U.V. | a. quantitative b. descending tech. | 383 |
| b. Coumarins | — | b. hexane-EtOAc (85:15) | b. 2,4-DNP-H c. o-dianisidine in glacial HOAc d. U.V. | c. monitored column d. diagnostic | 387 388 417 |
| c. Hydrocarbons | — | c. heptane to develop and elute hydrocarbons from end of layer | c. Fluorescein-Br ₂ | e. quant. assay of hydrocarbons by gas chromatography | 385 |
| d. Citral | — | d. hexane-EtOAc (85:15) | f. fluorescein-Br ₂ , viewed under U.V. | f. diagnostic | 386 |
| e. Chalcones | — | e. hexane-EtOAc (75:25) f. EtOEt-pet. ether (65:35) | g. HCl gas h. U.V. | g. diagnostic | 384 |
| 23 Oils of sage | Silica Gel G | benzene | vanillin in H ₂ SO ₄ | diagnostic | 49 |
| 25 Volatile oil of grafted umbelliferous plants | — | — | — | diagnostic | 198 199 |
| 26 Oils of <i>Curcumina</i> | Silica Gel G | benzene-CH ₂ Cl-MeOH (200:100:3) | SbCl ₃ in CH ₂ Cl ₂ | diagnostic | 445 |
| 27 Oils of <i>Anethus calamus</i> | Silica Gel G | benzene | — | a. diagnostic b. to relate plants | 453 |
| 28 Oils of <i>coriander lavender</i> and muscat sage | — | — | — | — | 298 |

| | | | | | | |
|----|--|---------------------------------|----------------------|--|------------|-----|
| 29 | Oils of <i>Oleinum rosmarinii</i> , <i>O. Lavandulae</i> and <i>O. roseae</i> | Silica Gel G | benzene-EtOAc (95:5) | a. SbCl ₃ in CHCl ₃ b. SbCl ₃ in CCl ₄ c. conc. H ₂ SO ₄ | diagnostic | 172 |
| 30 | 2,4-DNPH's of aldehydes and ketones from essential oils | see "Aldehydes, etc." No. 14 | | | | 209 |
| 31 | Removal of hydrocarbons from essential oils | see "Terpenoids," No. 1 | | | | 190 |

Thin-Layer Chromatography

TABLE 11.9. FLAVONOIDS AND COUMARINS

| Compound | Adsorbent | Developer | Visualization | Comments | Ref. |
|-------------------------------------|--|---|--|---|------------------|
| 1 Isoflavones | starch-bound silicic acid with phosphors | a. EtOAc-Skellysolve B (3:1) (1:1) b. acetone-EtOAc-Skellysolve B (4:3:3) c. EtOH-CHCl ₃ (1:3)(1:1) | U.V. | a. diagnostic b. preparative | 132 |
| 2 Flavonoids | unbound polyamide powder (Silion) | a. MeOH-H ₂ O (80:20)(60:40) b. MeOH | U.V. | diagnostic | 71 74 |
| 3 Flavonoids and coumarins | Silica Gel G-NaOAc | toluene-ethyl formate-formic acid (5:4:1) | diphenylboric acid, β -aminoethyl ester | diagnostic | 379 |
| 4 Flavonoids and coumarins of lemon | starch-bound silicic acid with phosphors | CHCl ₃ -acetone mixtures | U.V. | a. diagnostic b. monitored column | 162 |
| 5 Coumarins | Silica Gel G | a. pet. ether-EtOAc (2:1) b. hexane-EtOAc (5:2) | KOH and diazotized sulfanilic acid | diagnostic | 396 |
| 6 Coumarins in lemon juice | starch-bound silicic acid with phosphors | hexane-EtOAc (75:25) | U.V. | preparative | 31 |
| 7 Coumestrol in plants | starch-bound silicic acid with phosphors | a. CHCl ₃ -EtOEt (1:1) b. EtOAc-“Skellysolve” B (3:1) c. acetone-“Skellysolve” B (1:3) d. EtOEt-“Skellysolve” B (7:3) | U.V. | a. diagnostic b. quantitative d. diagnostic | 222 224 34 |

TABLE 11.10. INORGANIC AND METALORGANIC COMPOUNDS

| | Compound | Adsorbent | Developer | Visualization | Comments | Ref. |
|---|--|---|--|--|---|------|
| 1 | Fe ⁺² , Zn ⁺² , Be ⁺² , Ni ⁺² , and others | starch-bound alumina; "Celite" | a. H ₂ O b. dilute HCl c. organic liquids | a. I ₂ b. U.V. | a. diagnostic b. circular on microscope slides | 246 |
| 2 | a. Copper family-Cu ⁺² , Cd ⁺² , Bi ⁺³ , Pb ⁺² , Hg ⁺² b. Sulfide group-Zn ⁺² , Co ⁺² , Fe ⁺² , Ni ⁺² , Mn ⁺² , Cr ⁺³ , Al ⁺³ | purified Silica Gel G | a. BuOH-1.5 N HCl-2,5-hexanedione (100:20:0.5) b. acetone-conc. HCl-2,5-hexanedione (100:1:0.5) | a. KI b. NH ₃ and H ₂ S c. NH ₃ d. β-hydroxyquinoline e. U.V. | diagnostic theory | 359 |
| 3 | U ⁺⁶ , Ga ⁺³ and Al ⁺³ | purified Silica Gel G | a. EtOAc (sat. with H ₂ O)-tributyl phosphate (50:2) b. acetone-conc. HCl (100:0.5) | a. pyridyl-azo-naphthalol b. β-hydroxyquinoline c. U.V. | diagnostic | 360 |
| 4 | Alkali metals-Na ⁺¹ , Li ⁺¹ , K ⁺¹ , Mg ⁺² | purified Silica Gel G | EtOH-HOAc (100:1) | acid violet | diagnostic | 358 |
| 5 | Halogens-F ⁻¹ , Cl ⁻¹ , Br ⁻¹ , I ⁻¹ | purified Silica Gel G | acetone-BuOH-conc. NH ₄ OH-H ₂ O (65:20:10:5) | a. brom cresol purple and NH ₃ b. ammonical AgNO ₃ and fluorescein | diagnostic | 357 |
| 6 | Various phosphates | purified Silica Gel G | MeOH-conc. NH ₄ OH-10% trichloroacetic acid and H ₂ O (50:15:5:30) | ammonium molybdate followed by SnCl ₂ and HCl | diagnostic | 355 |
| 7 | K ⁺¹ and Mg ⁺² | Silica Gel G | EtOH-MeOH (1:1) plus HOAc | acid violet 6BN | quantitative | 315 |
| 8 | Radioactive halogens | "Dowex 1" ¹² Cellulose Powder MN 300 G (1:1) | 1 M aqueous NaNO ₃ | radioactivity | diagnostic | 29 |

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TABLE 11.9. FLAVONOIDS AND COUMARINS

| | Compound | Adsorbent | Developer | Visualization | Comments | Ref. |
|---|------------------------------------|--|--|---|---|------------------|
| 1 | Isoflavones | starch-bound silicic acid with phosphors | a. EtOAc-Skellysolve B (3:1) (1:1) b. acetone-EtOAc-Skellysolve B (4:3:3) c. EtOH-CHCl ₃ (1:3)(1:1) | U.V. | a. diagnostic b. preparative | 132 |
| 2 | Flavonoids | unbound polyamide powder (Silon) | a. MeOH-H ₂ O (80:20)(60:40) b. MeOH | U.V. | diagnostic | 71 74 |
| 3 | Flavonoids and coumarins | Silica Gel G-NaOAc | toluene-ethyl formate-formic acid (5:4:1) | diphenylboric acid, β -aminomethyl ester | diagnostic | 379 |
| 4 | Flavonoids and coumarins of lemon. | starch-bound silicic acid with phosphors | CHCl ₃ -acetone mixtures | U.V. | a. diagnostic b. monitored column | 162 |
| 5 | Coumarins | Silica Gel G | a. pet. ether-EtOAc (2:1) b. hexane-EtOAc (5:2) | KOH and diazotized sulfanilic acid | diagnostic | 396 |
| 6 | Coumarins in lemon juice | starch-bound silicic acid with phosphors | hexane-EtOAc (75:25) | U.V. | preparative | 31 |
| 7 | Coumestrol in plants | starch-bound silicic acid with phosphors | a. CHCl ₃ -EtOEt (1:1) b. EtOAc-Skellysolve ² B (3:1) c. acetone-Skellysolve ² B (1:3) d. EtOEt-Skellysolve ² B (7:3) | U.V. | a. diagnostic b. quantitative d. EtOEt-Skellysolve ² B (7:3) | 222 224 34 |

TABLE 11.10. INORGANIC AND METALORGANIC COMPOUNDS

| | Compound | Adsorbent | Developer | Visualization | Comments | Ref. |
|---|--|---|--|---|---|------------|
| 1 | Fe^{+2} , Zn^{+2} , Be^{+2} , Ni^{+2} , and others | starch-bound alumina, ^a "Celite" | a. H_2O b. dilute HCl c. organic liquids | a. I_2 b. U.V. | a. diagnostic b. circular on microscope slides | 246 |
| 2 | a. Copper family: Cu^{+2} , Cd^{+2} , Bi^{+3} , Pb^{+2} , Hg^{+2} b. Sulfide group: Zn^{+2} , Co^{+2} , Fe^{+3} , Ni^{+2} , Mn^{+2} , Cr^{+3} , Al^{+3} | purified Silica Gel G | a. $\text{BuOH}-1.5\text{N HCl}-2.5\text{-hexanone}$ (100:20:0.5) b. acetone-conc. HCl -2.5-hexanone (100:1:0.5) | a. KI b. NH_3 and H_2S c. NH_3 d. 8-hydroxyquinoline e. U.V. | diagnostic theory | 359 356 |
| 3 | U^{+6} , Ga^{+3} and Al^{+3} | purified Silica Gel G | a. EtOAc (sat. with H_2O)-tributyl phosphate (50:2) b. acetone-conc. HCl (100:0.5) | a. pyridyl-azo-naphthal b. 8-hydroxyquinoline c. U.V. | diagnostic | 360 |
| 4 | Alkali metals-Na ⁺ , Li ⁺ , K^{+1} , Mg^{+2} | purified Silica Gel G | EtOH-HOAc (100:1) | acid violet | diagnostic | 358 |
| 5 | Halogens-F ⁻¹ , Cl ⁻¹ , Br ⁻¹ , I ⁻¹ | purified Silica Gel G | acetone-BuOH-conc. NH_4OH - H_2O (65:20:10:5) | a. brom cresol purple and NH_3 b. ammonical AgNO_3 and fluorescein | diagnostic | 357 |
| 6 | Various phosphates | purified Silica Gel G | MeOH -conc. NH_4OH -10% trichloroacetic acid and H_2O (50:15:5:30) | ammonium molybdate followed by SnCl_4 and HCl | diagnostic | 355 |
| 7 | K^{+1} and Mg^{+2} | Silica Gel G | EtOH-MeOH (1:1) plus HOAc | acid violet 6BN | quantitative | 315 |
| 8 | Radioactive halogens | "Dowex 1" ^b -Cellulose Powder MN 300 G (1:1) | 1 M aqueous NaNO_3 | radioactivity | diagnostic | 29 |

TABLE II.10—Cont.

| | Compound | Adsorbent | Developer | Visualization | Comments | Ref. |
|----|--|---|--|--|------------|------|
| 9 | Twenty-four ferrocene derivatives | Silica Gel G | a. benzene b. benzene-EtOH (30:1)(15:1) c. propylene glycol-MeOH (1:1) d. propylene glycol-chlorobenzene-MeOH (1:1:1) | a. Br ₂ b. NaIO ₄ | diagnostic | 345 |
| 10 | Chromium and cobalt complexes of <i>o</i> -hydroxy- <i>o</i> '-carboxyazo dyes | Aluminum Oxide Fluka | MeOH | colored compounds | diagnostic | 342 |
| 11 | Organic stabilizers in polyvinyl chloride | Silica Gel G containing "Komplexon III" | H ₂ O-BuOH-EtOH-HOAc (10:5:5:0.15) | Diphenylthiocarbazone | diagnostic | 411 |

TABLE 11.11. INSECTICIDES

| | Compound | Adsorbent | Developer | Visualization | Comments | Ref. |
|---|---|------------------------------------|--|---|---|------|
| 1 | a. Thiophosphate esters b. Chlorinated compounds | Silica Gel D5 Aluminum Oxide D5 | hexane-acetone (4:1) hexane | PdCl ₂ in dilute HCl N,N-dimethyl- <i>p</i> -phenylenediamine hydrochloride in sodium methylate | a. diagnostic b. in forensic chemistry | 23 |
| 2 | Dichlorophene and hexachlorophene | starch-bound silicic acid | heptane saturated with HOAc | FeCl ₃ -K ₃ Fe(CN) ₆ | a. diagnostic b. quantitative | 43 |
| 3 | Thiophosphate esters | starch-bound aluminum oxide | heptane-acetone (10:1) | H ₂ IO ₆ -HClO ₄ -V ₂ O ₅ | diagnostic | 304 |
| 4 | Pyrethrins | plaster-of-Paris-bound silica gel | EtOAc-hexane (20:80) | fluorescein-Br ₂ | diagnostic | 365 |
| 5 | Pyrethrins and their peroxides | Silica Gel G | a. benzene-methyl ethyl ketone (90:10) b. benzene-EtOAc (85:15) c. CCl ₄ -EtOAc (80:20) d. hexane-methyl ethyl ketone (80:20) e. hexane-EtOAc (75:25) | SbCl ₃ in CHCl ₃ | a. diagnostic b. reaction on layer | 371 |
| 6 | Pentachlorophenol | Silica Gel G-oxalic acid | CHCl ₃ | U.V. | a. diagnostic b. quantitative | 83 |
| 7 | Chlorinated organic pesticides | | | | | 455 |
| 8 | Chlorinated hydrocarbons with insecticidal properties | | | | | 300 |

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TABLE 11.12. LIPIDS—CLASS SEPARATION

| | Compound | Absorbent | Developer | Visualization | Comments | Ref. |
|---|-----------------------------|--|--|---|--|------|
| 1 | Misc. lipids | Silica Gel G | a. EtOEt b. isopropyl ether c. isopropyl ether-HOAc (98:5:1.5) | Phosphomolybdic acid | Class separation prior to analysis by reversed phase technique | 182 |
| 2 | Misc. lipids | Silica Gel G | pet. ether-EtOEt-HOAc (90:10:1) | 2',7'-dichlorofluorescein | Class separation prior to analysis by reversed phase technique | 230 |
| 3 | Misc. lipids | | | | monitored column | 150 |
| 4 | Radioactive lipids | silicic acid | pet. ether-EtOEt (92:8) | a. radioactivity b. α -cyclodextrin-I ₂ c. I ₂ | class separation | 231 |
| 5 | Industrial aliphatic lipids | a. Silica Gel G b. benzene sat. at 20° with 1 N NH ₄ OH c. CHCl ₃ (sat. with 1 N NH ₄ OH)-MeOH (97:3) d. acetone-14 N NH ₄ OH (90:10) | a. pet. ether-benzene (95:5) b. 2',7'-dichlorofluorescein c. K ₂ Cr ₂ O ₇ in H ₂ SO ₄ | a. I ₂ b. 2',7'-dichlorofluorescein c. K ₂ Cr ₂ O ₇ in H ₂ SO ₄ | diagnostic | 234 |
| | b. Silica Gel G | e. CHCl ₃ -(MeOH-0.1 N H ₂ SO ₄ , 95:5)(97:3)(80:20) | | | | |
| 6 | Misc. fats, oils and waxes | Silica Gel G | a. pet. ether-EtOEt-HOAc (90:10:1)(70:30:2)(70:30:1) b. hexane-EtOEt (95:5) | a. I ₂ b. 2',7'-dichlorofluorescein | a. diagnostic b. class separation | 235 |
| 7 | Tissue lipids | Silica Gel G | hexane-EtOEt-HOAc (90:10:1) | 2',7'-dichlorofluorescein | class separation prior to assay by gas chromatography | 236 |
| 8 | Serum lipids | unbound aluminum oxide | a. pet. ether-EtOEt (95:5) b. pet. ether-EtOH (98:2) c. pet. ether-EtOEt-HOAc (94:5:5:0.5) d. heptane-HOAc (98:2) | | a. class separation b. quantitative | 413 |

| | | | | | | |
|----|---|-------------------------------------|--|--|--|------------------|
| 9 | Serum lipids and phospholipids | Plaster-of-Paris-bound silica acid. | a. $\text{CHCl}_3\text{-MeOH-H}_2\text{O}$ (80:25:3) b. pet. ether-EtOEt-H ₂ OAc (90:10:1)(60:40:1) | $2',7'$ -dichlorofluorescein | diagnostic | 423 |
| 10 | Serum lipids | Silica Gel G | a. PrOH-NH ₄ OH (2:1)-1st step b. $\text{CHCl}_3\text{-benzene}$ (3:2) 2nd step c. CCl_4 -reverse | SbCl ₃ in CHCl_3 | a. stepwise technique b. two dimensional c. quantitative | 21 164 438 |
| 11 | Serum, liver and adipose tissue lipids | Silica Gel G | | | | 86 |
| 12 | Plasma lipids | Silica Gel G | $\text{CHCl}_3\text{-MeOH-H}_2\text{O}$ (65:25:4) | a. bromthymol blue-NH ₃ b. ninhydrin c. fuchsin-H ₂ SO ₄ d. hydroxamate reaction | a. diagnostic b. quantitative for phosphates | 134 |
| 13 | Plasma lipids | Silica Gel G | a. pet. ether-diisobutylketone-HOAc (87:13:0.7) b. pet. ether-methyl ethyl ketone-HOAc (95:4:1) | a. 50% H ₂ SO ₄ b. Rhodamine 6 G c. bromthymol blue d. osmium tetroxide | class separation prior to separation by reversed phase technique | 459 |
| 14 | Butter-fat lipid | | | | | 245 |
| 15 | Brain tissue lipids | Silica Gel G | a. $\text{CHCl}_3\text{-MeOH-H}_2\text{O}$ (24:7:1) b. CHCl_3 c. CCl_4 | a. ammonium molybdate-HClO ₄ b. phosphomolybdic acid c. ninhydrin d. Dragendorff's reagent e. diphenylamine | diagnostic on multiple sclerosis patients | 157 |
| 16 | Brain lipid constituents (pure test substances) | Silica Gel G | a. heptane b. CCl_4 c. 1,2-dichloroethane d. CHCl_3 e. 1,2-dichloroethane-MeOH (98:2) f. pet. ether-EtOEt (70:30) g. four others | a. bromthymol blue, buffered b. bromthymol blue c. conc. H ₂ SO ₄ -HOAc (1:1) d. phosphomolybdic acid e. HIO ₄ -Schiff reagent f. aqua regia-ammonium molybdate-vanadyl chloride g. diphenylamine | a. diagnostic b. system for exploring unknown lipids | 174 |

TABLE II.12—*Cont.*

| | Compound | Adsorbent | Developer | Visualization | Comments | Ref. |
|----|------------------------------------|--------------|---------------------------------|------------------------------------|--|------------|
| 17 | Dogfish liver lipids | Silica Gel G | pet. ether-EtOEt-HOAc (90:10:1) | | separated the alkoxy-glycerides class for assay | 229 |
| 18 | Lipids of feces and fecal- iths | Silica Gel G | pet. ether-EtOEt-HOAc (80:20:1) | 50% H ₂ SO ₄ | a. class separation b. semiquantitative assay | 442 |
| 19 | Lipids | | | | review review | 104 232 |

TABLE 11.13. LIPIDS—FATTY ACIDS AND THEIR DERIVATIVES

| | Compound | Absorbent | Developer | Visualization | Comments | Ref. |
|---|--|---|--|---|---|------|
| 1 | Fatty acids | plaster of Paris impregnated with undecane | a. HOAc-CH ₃ CN (1:1) b. solution a with 0.5% Br ₂ | copper acetate-dithiooxamide | separated "critical" pairs after hydrogenation and bromination on layer | 180 |
| 2 | Fatty acids | Kieselguhr G impregnated with undecane | HOAc-CH ₃ CN (1:1) | copper acetate-dithiooxamide on layers "smoked" with dichlorodimethylsilane | diagnostic | 185 |
| 3 | Fatty acids | Kieselguhr G impregnated with undecane | a. HOAc-CH ₃ CN (6:4), 90% sat. with undecane b. HOAc-CH ₃ CN (30:70), 80% sat. with undecane and containing 0.5% Br ₂ | Rhodamine B | separated "critical" pairs after hydrogenation and bromination on layer | 186 |
| 4 | Fatty acids | Silica Gel G impregnated with undecane | a. 96% HOAc b. HOAc-CH ₃ CN (1:1) | phosphomolybdic acid | diagnostic | 182 |
| 5 | Fatty acids of Swiss Pharmacopoeia | see "Lipids-Glycerides", No. 16 | | | | 6 |
| 6 | Keto- and hydroxy-fatty acids and their lactones | Kieselguhr G impregnated with tetradecane or paraffin | a. HOAc-H ₂ O (80:20), 80% sat. with impregnating solution (5% of agent in pet. ether) b. HOAc-H ₂ O (90:10), 75% sat. with impregnating solution | a. Rhodamine B b. phosphomolybdic acid | diagnostic | 181 |
| 7 | Fatty acids of castor oil and oiticica oil | Silica Gel G | pet. ether-EtOEt-HOAc (70:30:1)(70:30:2) | 2',7'-dichlorofluorescein | diagnostic | 235 |
| 8 | Lignoceric, cerebronic and nervonic acids | Silica Gel G | | | diagnostic | 109 |
| 9 | Fatty acid methyl esters | starch-bound silicic acid with phosphors | a. "Skellysolve" F-EtOEt (90:10)(70:30)(50:50) b. benzene-EtOEt (75:25) c. fluorescein-Br ₂ | a. 2',7'-dichlorofluorescein b. U.V. c. fluorescein-Br ₂ | a. diagnostic b. monitored column c. U.V. | 8 |

TABLE 11.13—Cont.

| | Compound | Adsorbent | Developer | Visualization | Comments | Ref. |
|----|----------------------------------|--|---|--|---|------|
| 10 | Fatty acid methyl esters | Silica Gel G impregnated with AgNO_3 | EtOEt-hexane (10:90)(40:60) | 2',7'-dichloro-fluorescein | diagnostic | 260 |
| 11 | Fatty acid methyl esters | Silica Gel G | hexane-EtOEt-HOAc (85:15:1) | 2',7'-dichloro-fluorescein | preparative separation prior to analysis by gas chromatog. | 333 |
| 12 | Fatty acid methyl esters | Silica Gel G | hexane-EtOEt mixtures (up to 30% EtOEt) | a. I ₂ b. 2',7'-dichloro-fluorescein c. 50% H_2SO_4 | a. quantitative b. preparative | 420 |
| 13 | Fatty acid methyl esters | Silica Gel G impregnated with Silicone oil | a. $\text{CH}_3\text{CN}-\text{HOAc}-\text{H}_2\text{O}$ (70:10:25) b. peracetic acid-HOAc-H ₂ O (10:75:15) | a. I ₂ α -cyclodextrin and I ₂ b. solution a plus 1% HOAc | a. diagnostic b. separated sat. esters by using an oxidizing developer | 230 |
| 14 | Fatty acid methyl esters | Silica Gel G | a. pet. ether-EtOEt (80:20) b. solution a plus 1% HOAc | 50% H_2SO_4 | diagnostic | 262 |
| 15 | Epoxy fatty acid methyl esters | Silica Gel G | hexane-EtOEt (95:5)(90:10) | 50% H_2SO_4 | monitored various separation methods | 261 |
| 16 | Fatty acids and esters | silicic acid on the inside of test tubes | a. hexane b. hexane-EtOEt (97:3)(85:15) c. CHCl_3 | I ₂ | diagnostic | 219 |
| 17 | Fatty acid esters of orujo oil | Silica Gel G | hexane-EtOEt (90:10) | 50% H_2SO_4 | a. diagnostic b. monitored column | 422 |
| 18 | Fatty acid esters of cholesterol | a. Silica Gel G b. Silica Gel G impregnated with paraffin | a. tetralin-hexane (25:75)(1:1) b. CCl_4 c. methyl ethyl ketone- CH_3CN (7:3) | phosphomolybdic acid | a. diagnostic b. two-dimension | 184 |

| | | | | | | |
|----|---|----------------------------------|--|--|--|------------|
| 19 | Fatty acids and their cholesterol esters | Silica Gel G | a. PrOH-NH ₂ OH (2:1) b. CHCl ₃ -benzene (3:2) c. CCl ₄ | SbCl ₃ in CHCl ₃ | a. diagnostic b. stepwise technique c. two-dimension | 438 |
| 20 | Cholesterol and its esters | see "Steroids," No 28 and No. 27 | | | | 228 249 |
| 21 | Acetoxymercurymethoxy derivatives of unsaturated fatty acid methyl esters | Silica Gel G | a. pet. ether-EtOEt (4:1) b. PrOH-HOAc (100:1) | s-diphenylcarbazone | isolated unsaturated esters prior to assay by gas chromatography | 177 233 |
| 22 | p-Phenylazophenacyl esters of fatty acids | Silica Gel G | pet. ether-EtOEt (85:15) | colored compounds | diagnostic | 419 |

TABLE 11.14. LIPIDS—GLYCERIDES

| Compound | Adsorbent | Developer | Visualization | Comments | Ref. |
|------------------|---|---|--|--|----------------|
| 1 Glycerides | Silica Gel G impregnated with AgNO_3 | $\text{CHCl}_3\text{-HOAc}$ (99.5:0.5) | dibromo-R-fluorescein | separation according to degree of unsaturation | 16 |
| 2 Glycerides | Silica Gel G | $\text{CHCl}_3\text{-benzene}$ (70:30) | conc. H_2SO_4 | separation of the glyceride fraction from other fractions | 64 |
| 3 Glycerides | Silica Gel G impregnated with undecane | a. $\text{CHCl}_3\text{-MeOH-H}_2\text{O}$ (5:15:1) for diglycerides b. acetone- CH_3CN (7:3) for triglycerides | Rhodamine B | diagnostic | 182 |
| 4 Glycerides | Silica Gel G | a. "Skellysolve" F-EtOE (70:30) b. (10:90) for monoglycerides c. (70:30) for diglycerides d. (90:10) for triglycerides e. (60:40)(35:65)(85:15) | 50% H_2SO_4 | a. quantitative b. quantitative based upon ozonolysis and periodate oxidation | 311 310 |
| 5 Glycerides | plaster-of-Paris-bound silicic acid | gradient elution system of pet. ether-EtOE varied between (9:1) and (4:6). All contained 0.1% HOAc. | 2',7'-dichlorofluorescein | a. diagnostic b. gradient elution | 334 |
| 6 Glycerides | Silica Gel G | a. 1,2-dichloroethane b. CHCl_3 c. 1,2-dichloroethane-MeOH (98:2) d. pet. ether-EtOE (70:30) e. $\text{CHCl}_3\text{-HOAc}$ (96:4) | a. HIO_4 -Schiff reagent b. bromthymol blue | diagnostic | 174 |
| 7 Monoglycerides | hydroxyl-apatite | methyl isobutyl ketone at 10° | phosphomolybdic acid | diagnostic | 155 |
| 8 Triglycerides | Kieselguhr G impregnated with petroleum | acetone- CH_3CN (8:2)(7:4) | a. phosphomolybdic acid b. I ₂ c. α -cyclodextrin-I ₂ | a. diagnostic b. multiple development | 179 |

| | | | | | | |
|----|---|--|---|--|---|-----|
| 9 | Triglycerides | plaster of Paris impregnated with paraffin (b.p. 240-250°) | a. acetone-CH ₃ CN (8:2) b. propanoic acid-CH ₃ CN (6:4) plus 0.5% Br ₂ by vol. | Sudan Black B | a. diagnostic b. bromination on layers | 180 |
| 10 | Triglycerides | Kieselguhr G impregnated with tetradecane | a. acetone-CH ₃ CN (8:2) 80% sat. with tetradecane b. propanoic acid-CH ₃ CN (6:4) 80% sat. with tetradecane plus 0.5% Br ₂ by vol. | a. I ₂ b. phosphomolybdic acid | a. diagnostic b. bromination on layer | 186 |
| | | Kieselguhr G impregnated with paraffin (b.p. 240-250°) or Silicone oil | c. acetone-CH ₃ CN (8:2) d. MeOH-CH ₃ CN (5:4) e. MeOH-CH ₃ CN-CH ₃ CH ₂ CN (5:4:1.5) f. solution e saturated with silicone oil | c. Rhodamine B d. I ₂ e. phosphomolybdic acid | diagnostic | 183 |
| 11 | Glycerides of milk fat (lipolyzed) | Silica Gel G | pet. ether-EtOH-HOAc (90:10:1) | | preparative separation prior to assay by gas chromatography | 178 |
| 12 | Glycerides of linseed oil | plaster-of-Paris-bound silicic acid | gradient elution from pet. ether to pet. ether-EtOEt (1:1) | conc. H ₂ SO ₄ | a. diagnostic b. quantitative c. used to find optimum reaction conditions | 335 |
| 13 | C ¹⁴ and tritium assay of triphosphoinositidyl fatty acids | Silica Gel G | pet. ether-EtOEt-HOAc (90:10:1) | a. I ₂ b. 2',7'-dichlorofluorescein | a. quantitative b. preparative | 364 |
| 14 | Alkoxylglycerides | | | | diagnostic | 212 |
| 15 | Acetoglycerides | silicic acid | "Skellysolve" F-EtOEt (92:8) | I ₂ | diagnostic | 130 |
| 16 | Glycerides of the Swiss Pharmacopoeia | Kieselguhr G impregnated with paraffin HOAc | | a. I ₂ and benzidine b. Rhodamine B and KOH | diagnostic | 6 |
| 17 | Glycerides from plasma | Silica Gel G | pet. ether-methyl ethyl ketone-HOAc (8:15:1) | a. 50% H ₂ SO ₄ b. osmium tetroxide c. Rhodamine 6 G | diagnostic | 459 |

TABLE II.15. LIPIDS—GLYCOLIPIDS

| Compound | Adsorbent | Developer | Visualization | Comments | | Ref. |
|------------------|--------------|---|---|---|---|------|
| | | | | a. diphenylamine | a. diagnostic b. stepwise tech- nique | |
| 1 Glycolipids | Silica Gel G | a. PrOH-NH ₄ OH (12% NH ₃)(4:1) b. 1,2-dichloroethane-MeOH (49:1) c. CHCl ₃ -96% HOAc (95:5) all consecutively | diphenylamine | a. diagnostic b. stepwise tech- nique | 174 | |
| 2 Glycolipids | Silica Gel G | CHCl ₃ -MeOH-H ₂ O (60:35:8) (65:25:4) | a. Rhodamine B b. Dragendorff's reag. c. Bial's reagent d. diphenylamine | diagnostic | 427 432 | |

TABLE 11.16. LIPIDS—PHOSPHOLIPIDS AND SULFOLIPIDS

| | Compound | Adsorbent | Developer | Visualization | Comments | Ref. |
|----|--|--|--|---|--------------|------------|
| 1 | Cephalins and lecithins | Silica Gel G | $\text{CHCl}_3\text{-MeOH-H}_2\text{O}$ (11:5:1) | phosphomolybdc acid-SnCl ₂ on layer "smoked" with dichlorodimethylsilane | diagnostic | 185 |
| 2 | Sphingosines, etc. | silicic acid on walls of test tubes | a. CHCl_3 b. $\text{NH}_3\text{-MeOH-CHCl}_3$ (20:80) (30:70) ($\text{NH}_3\text{-MeOH}$ is $\text{MeOH-H}_2\text{O-NH}_3\text{OH}$ (28% NH_3) (93:5:2)) | I_2 | diagnostic | 219 |
| 3 | Lecithin and colaminecephalin | Silica Gel G | $\text{CHCl}_3\text{-MeOH-H}_2\text{O}$ (65:25:4) | a. Rhodamine B b. Dragendorff's reag. c. ninhydrin | quantitative | 430 |
| 4 | Misc. phospholipids | Silica Gel G | $\text{CHCl}_3\text{-MeOH-H}_2\text{O}$ (60:35:8) (65:25:4) | a. Rhodamine B b. Dragendorff's reag. c. ammonium molybdate- HClO_4 d. ninhydrin | diagnostic | 427 432 |
| 5 | Serum phospholipids | plaster-of-Paris-bound silicic acid | $\text{CHCl}_3\text{-MeOH-H}_2\text{O}$ (80:25:3) | $2',7'$ -dichlorofluorescein | diagnostic | 423 |
| 6 | Lecithins, cephalins and sphingomyelins | Silica Gel G with 10% $(\text{NH}_4)_2\text{SO}_4$ | $\text{CHCl}_3\text{-H}_2\text{SO}_4\text{-MeOH}$ (80:20) $(\text{H}_2\text{SO}_4\text{-MeOH}$ is 0.1 N aqueous $\text{H}_2\text{SO}_4\text{-MeOH}$ (5:95)) | a. I_2 b. $2',7'$ -dichlorofluorescein c. $\text{K}_3\text{Cr}_2\text{O}_7\text{-H}_2\text{SO}_4$ | diagnostic | 234 |
| 7 | Cerebrin, kerasin and their sulfuric acid esters | Silica Gel G | a. 1,2-dichloroethane b. $\text{CHCl}_3\text{-HOAc}$ (96:4) c. $\text{PrOH-NH}_3\text{OH}$ (12.5% NH_3) (8:2) | bromthymol blue | diagnostic | 173 |
| 8 | Beef brain gangliosides | Silica Gel G | $\text{PrOH-H}_2\text{O}$ (7:3) | a. bromthymol blue b. Ehrlich reagent | preparative | 208 |
| 9 | Cerebral gangliosides | Silica Gel G | $\text{BuOH-pyridine-H}_2\text{O}$ (3:2:1) | Bial's reagent | diagnostic | 197 |
| 10 | Beef brain gangliosides | Silica Gel G | $\text{PrOH-1}N\text{ NH}_3\text{OH-H}_2\text{O}$ (6:2:1) | $\text{PrOH-1}N\text{ NH}_3\text{OH-H}_2\text{O}$ (6:2:1) | diagnostic | 440 |

TABLE 11.15. LIPIDS—GLYCOLIPIDS

| | Compound | Adsorbent | Developer | Visualization | Comments | Ref. | |
|---|-------------|--------------|---|---|------------|------------|-----|
| 1 | Glycolipids | Silica Gel G | a. PrOH:NH ₄ OH (12% NH ₃)(4:1) b. 1,2-dichloroethane-MeOH (49:1) c. CHCl ₃ -96% HOAc (95:5) all consecutively | diphenylamine | | | 174 |
| | | | | a. diagnostic b. stepwise tech- nique | | | |
| | | | | | | | |
| 2 | Glycolipids | Silica Gel G | CHCl ₃ -MeOH-H ₂ O (60:35:8) (65:25:4) | a. Rhodamine B b. Dragendorff's reag. c. Bial's reagent d. diphenylamine | diagnostic | 427 432 | |

TABLE 11.16. LIPIDS—PHOSPHOLIPIDS AND SULFOLIPIDS

| | Compound | Adsorbent | Developer | Visualization | Comments | Ref. |
|----|--|---|---|---|--------------|------------|
| 1 | Cephalins and lecithins | Silica Gel G | CHCl ₃ -MeOH-H ₂ O (11:5:1) | phosphomolybdic acid-SnCl ₂ on layer, "smoked" with dichlorodimethylsilane | diagnostic | 185 |
| 2 | Sphingosines, etc. | silicic acid on walls of test tubes | a. CHCl ₃ b. NH ₃ /MeOH-CHCl ₃ (20:80) (30:70) (NH ₃ /MeOH is MeOH-H ₂ O-NH ₃ OH (38% NH ₃) (33:5:2)) | I ₂ | diagnostic | 219 |
| 3 | Lecithin and colaminecephalin | Silica Gel G | CHCl ₃ -MeOH-H ₂ O (65:25:4) | a. Rhodamine B b. Dragendorff's reag. c. ninhydrin | quantitative | 430 |
| 4 | Misc. phospholipids | Silica Gel G | CHCl ₃ -MeOH-H ₂ O (60:35:8) (65:25:4) | a. Rhodamine B b. Dragendorff's reag. c. ammonium molybdate-HClO ₄ d. ninhydrin | diagnostic | 427 432 |
| 5 | Serum phospholipids | plaster-of-Paris-bound silicic acid | CHCl ₃ -MeOH-H ₂ O (80:25:3) | 2',7'-dichlorofluorescein | diagnostic | 423 |
| 6 | Lecithins, cephalins and sphingomyelins | Silica Gel G with 10% (NH ₄) ₂ SO ₄ | CHCl ₃ -H ₂ SO ₄ /MeOH (80:20) (H ₂ SO ₄ /MeOH is 0.1 N aqueous H ₂ SO ₄ -MeOH (5:95)) | a. I ₂ b. 2',7'-dichlorofluorescein c. K ₂ Cr ₂ O ₇ -H ₂ SO ₄ | diagnostic | 234 |
| 7 | Cerebrin, kerasin and their sulfuric acid esters | Silica Gel G | a. 1,2-dichloroethane b. CHCl ₃ -HOAc (96:4) c. P ₂ O ₅ -NH ₄ OH (12.5% NH ₃) (8:2) | bromthymol blue | diagnostic | 173 |
| 8 | Beef brain gangliosides | Silica Gel G | PrOH-H ₂ O (7:3) | a. bromthymol blue b. Ehrlich reagent | preparative | 208 |
| 9 | Cerebral gangliosides | Silica Gel G | BuOH-pyridine-H ₂ O (3:2:1) | Bial's reagent | diagnostic | 197 |
| 10 | Beef brain gangliosides | Silica Gel G | PrOH-1 <i>N</i> NH ₄ OH-H ₂ O (6:2:1) | | diagnostic | 440 |

TABLE II.17. NUCLEOTIDES

| | Compound | Adsorbent | Developer | Visualization | Comments | Ref. |
|---|--|---|---|---------------|---|------------|
| 1 | Misc. nucleotides | collodion-bound "ECT-EOLA" cellulose | a. 0.15 M NaCl b. 0.01 N HCl | U.V. | diagnostic | 316 317 |
| 2 | Misc. nucleotides | Cellulose Powder MN 300 G | a. H ₂ O b. sat. aqueous (NH ₄) ₂ SO ₄ -1 M NaOAc- 2-PrOH (90:18:2) c. <i>t</i> -amyl alcohol-formic acid-H ₂ O (3:2:1) d. BuOH-acetone-HOAc-NH ₄ OH (5% NH ₃)-H ₂ O (3:5:2:5:1:5:1:1) | U.V. | a. diagnostic b. comparison with paper chromatog. | 319 |
| 3 | Misc. nucleotides | a. DEAE-cellulose b. cellulose c. ECTEOLA-cellulose | dilute aqueous HCl 0.02 to 0.04 N sat. aqueous (NH ₄) ₂ SO ₄ -1 M NaOAc-2- PrOH (80:18:2) 0.06 N HCl | U.V. | a. diagnostic b. preparative c. review | 320 318 |
| 4 | Misc. nucleotides | Cellulose Powder MN 300 | a. H ₂ O for nucleobases b. BuOH-acetone-HOAc-NH ₄ OH (5% NH ₃)-H ₂ O (4:5:1.5:1:1:2) | U.V. | diagnostic | 321 |
| 5 | Nucleotides, AMP, ADP and ATP | "DEAE-Sephadex" A-25 | gradient elution starting with 1.0 N formic acid and adding 10 N formic acid which is 2 M in ammonium formate | fluorescein | diagnostic | 82 |
| 6 | Amino acid esters as models for amino- acrylribonucleic acid | see "Proteins, etc." No. 18 | | | | 456 |

TABLE 11.18. PHENOLS, ALCOHOLS AND THEIR DERIVATIVES

| | Compound | Adsorbent | Developer | Visualization | Comments | Ref. |
|----|---------------------------------------|---|--|---|---------------------------------|------|
| 1 | Acetylene alcohols and glycols | | | | | 4 |
| 2 | Chimyl, batyl and se-lachyl alcohols | see "Lipids-Glycerides," No. 14 | | | | 212 |
| 3 | Long-chain aliphatic alcohols | Silica Gel G | a. pet. ether-EtOEt-HOAc (90:10:1) b. pet. ether-EtOEt (20:80) (10:90)(70:30) | 50% H ₂ SO ₄ | diagnostic | 395 |
| 4 | Cyclohexanol derivatives | Silica Gel G | a. benzene b. benzene-EtOEt (80:20) | H ₂ O | a. diagnostic b. preparative | 128 |
| 5 | Cardanol and its methylol derivatives | plaster-of-Paris-bound silica gel | EtOAc-toluene (60:40) | diazotized sulfanilic acid | followed course of reaction | 11 |
| 6 | Phenolic acids and esters | see "Carboxylic Acids, etc., No. 9 | | | | 69 |
| 7 | Phenols | a. starch-bound silicic acid b. starch-bound silicic acid kieselguhr (1:1) | a. xylene b. xylene-CHCl ₃ (3:1)(1:1)(1:3) c. CHCl ₃ | diazotized sulfanilic acid followed by KOH | diagnostic | 426 |
| 8 | Pyrocatechol and enge-nol derivatives | Silica Gel G, acidic and basic | benzene | SB ₂ Cl ₆ in CCl ₄ | diagnostic | 369 |
| 9 | Phenols and phenolic acids | starch-bound silicic acid with phosphors | a. "Skellysolve" B-EtOEt (3:7) b. "Skellysolve" B-EtOAc (1:3) c. "Skellysolve" B-acetone (3:1) | U.V. | followed course of reaction | 225 |
| 10 | Phloroglucinol derivatives | see "Miscellaneous," No. 15 | | | | 380 |
| 11 | Phenolic aldehydes | see "Aldehydes, etc., No. 9 | | | | 396 |

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TABLE II.17. NUCLEOTIDES

| | Compound | Adsorbent | Developer | Visualization | Comments | Ref. |
|---|---|---|---|---------------|---|----------------|
| 1 | Misc. nucleotides | collodion-bound "ECT-EOLA" cellulose | a. 0.15 M NaCl b. 0.01 N HCl | U.V. | diagnostic | 316 317 |
| 2 | Misc. nucleotides | Cellulose Powder 300 G | MN a. H ₂ O b. sat. aqueous (NH ₄) ₂ SO ₄ -1 M NaOAc- 2-PrOH (80:18:2) c. <i>t</i> -amyl alcohol-formic acid-H ₂ O (3:2:1) d. BuOH-acetone-HOAc-NH ₄ OH (5% NH ₃)-H ₂ O (3:5:2.5:1.5:1.5:1) | U.V. | a. diagnostic b. comparison with paper chromatog. | 319 |
| 3 | Misc. nucleotides | a. DEAE-cellulose b. cellulose c. ECTEOLA-cellulose | dilute aqueous HCl 0.02 to 0.04 N sat. aqueous (NH ₄) ₂ SO ₄ -1 M NaOAc-2- PrOH (80:18:2) 0.06 N HCl | U.V. | a. diagnostic b. preparative c. review | 320 318 |
| 4 | Misc. nucleotides | Cellulose Powder MN 300 | a. H ₂ O for nucleobases b. BuOH-acetone-HOAc-NH ₄ OH (5% NH ₃)-H ₂ O (4:5:1.5:1:1:2) | U.V. | diagnostic | 321 |
| 5 | Nucleotides, AMP, ADP and ATP | "DEAE-Sephadex" A-25 | gradient elution starting with 1.0 N formic acid and adding 10 N formic acid which is 2 M in ammonium formate | fluorescein | diagnostic | 82 |
| 6 | Amino acid esters as models for amino- acylribonucleic acid | see "Proteins, etc., No. 18 | | | | 456 |

TABLE 11.18. PHENOLS, ALCOHOLS AND THEIR DERIVATIVES

| | Compound | Absorbent | Developer | Visualization | Comments | Ref. |
|----|---------------------------------------|---|--|--|---------------------------------|------|
| 1 | Acetylene alcohols and glycols | | | | | 4 |
| 2 | Chimyl, bayl and se-lachyl alcohols | see "Lipids-Glycerides," No. 14 | | | | 212 |
| 3 | Long-chain aliphatic alcohols | Silica Gel G | a. pet. ether-EtOEt-HOAc (90:10:1) b. pet. ether-EtOEt (20:80) (10:90)(70:30) | 50% H ₂ SO ₄ | diagnostic | 395 |
| 4 | Cyclohexanol derivatives | Silica Gel G | a. benzene b. benzene-EtOEt (80:20) | H ₂ O | a. diagnostic b. preparative | 128 |
| 5 | Cardanol and its methylol derivatives | Plaster-of-Paris-bound silica gel | EtOAc-toluene (60:40) | diazotized sulfanilic acid | followed course of reaction | 11 |
| 6 | Phenolic acids and esters etc., No. 9 | see "Carboxylic Acids, etc., No. 9 | | | | 69 |
| 7 | Phenols | a. starch-bound silicic acid b. starch-bound silicic acid kieselguhr (1:1) | a. xylene b. xylene-CHCl ₃ (3:1)(1:1)(1:1) c. CHCl ₃ | diazotized sulfanilic acid followed by KOH | diagnostic | 426 |
| 8 | Pyrocatechol and eugenol derivatives | Silica Gel G, acidic and basic | | SbCl ₃ in CCl ₄ | diagnostic | 369 |
| 9 | Phenols and phenolic acids | starch-bound silicic acid with phosphors | a. "Skellysolve" B-EtOEt (3:1:7) b. "Skellysolve" B-EtOAc (1:3) c. "Skellysolve" B-acetone (3:1) | U.V. | followed course of reaction | 225 |
| 10 | Phloroglucinol derivatives | see "Miscellaneous," No. 15 | | | | 380 |
| 11 | Phenolic aldehydes | see "Aldehydes, etc., No. 9 | | | | 396 |

Thin-Layer Chromatography

TABLE 11.17. NUCLEOTIDES

| | Compound | Adsorbent | Developer | Visualization | Comments | Ref. |
|---|--|---|---|---------------|---|----------------|
| 1 | Misc. nucleotides | collodion-bound "ECT-EOLA" cellulose | a. 0.15 M NaCl b. 0.01 N HCl | U.V. | diagnostic | 316 317 |
| 2 | Misc. nucleotides | Cellulose Powder MN 300 G | a. H ₂ O b. sat. aqueous (NH ₄) ₂ SO ₄ -1 M NaOAc- 2-PrOH (80:18:2) c. t-amyl alcohol-formic acid-H ₂ O (3:2:1) d. BuOH-acetone-HOAc-NH ₄ OH (5% NH ₃)-H ₂ O (3.5:2.5:1.5:1.5:1) | U.V. | a. diagnostic b. comparison with paper chromatog. | 319 |
| 3 | Misc. nucleotides | a. DEAE-cellulose b. cellulose c. EGTEOLA-cellulose | dilute aqueous HCl 0.02 to 0.04 N sat. aqueous (NH ₄) ₂ SO ₄ -1 M NaOAc-2- PrOH (80:18:2) 0.06 N HCl | U.V. | a. diagnostic b. preparative c. review | 320 318 |
| 4 | Misc. nucleotides | Cellulose Powder MN 300 | a. H ₂ O for nucleobases b. BuOH-acetone-HOAc-NH ₄ OH (5% NH ₃)-H ₂ O (4.5:1.5:1:1:2) | U.V. | diagnostic | 321 |
| 5 | Nucleotides, AMP, ADP and ATP | "DEAE-Sephadex" A-25 | gradient elution starting with 1.0 N formic acid and adding 10 N formic acid which is 2 M in ammonium formate | fluorescein | diagnostic | 82 |
| 6 | Amino acid esters as models for amino- acyribonucleic acid | see "Proteins, etc.," No. 18 | | | | 456 |

TABLE 11.18. PHENOLS, ALCOHOLS AND THEIR DERIVATIVES

| | Compound | Adsorbent | Developer | Visualization | Comments | Ref. |
|----|---------------------------------------|---|--|--|---------------------------------|------|
| 1 | Acetylene alcohols and glycols | | | | | 4 |
| 2 | Chimyl, baylyl and se-lachyl alcohols | see "Lipids-Glycerides," No. 14 | | | | 212 |
| 3 | Long-chain aliphatic alcohols | Silica Gel G | a. pet. ether-EtOEt-HOAc (90:10:1) b. pet. ether-EtOEt (20:80) (10:90)(70:30) | 50% H ₂ SO ₄ | diagnostic | 395 |
| 4 | Cyclohexanol derivatives | Silica Gel G | a. benzene b. benzene-EtOEt (80:20) | H ₂ O | a. diagnostic b. preparative | 128 |
| 5 | Cardanol and its methylol derivatives | plaster-of-Paris-bound silica gel | EtOAc-toluene (60:40) | diazotized sulfanilic acid | followed course of reaction | 11 |
| 6 | Phenolic acids and esters | see "Carboxylic Acids, etc.," No. 9 | | | | 69 |
| 7 | Phenols | a. starch-bound silicic acid b. starch-bound silicic acid kieselguhr (1:1) | a. xylene b. xylene-CHCl ₃ (3:1)(1:1)(1:3) c. CHCl ₃ | diazotized sulfanilic acid followed by KOH | diagnostic | 426 |
| 8 | Pyrocatechol and eugenol derivatives | Silica Gel G, acidic and basic | benzene | SbCl ₃ in CCl ₄ | diagnostic | 369 |
| 9 | Phenols and phenolic acids | starch-bound silicic acid with phosphors | a. "Skellysolve" B-EtOEt (3:7) b. "Skellysolve" B-EtOAc (1:3) c. "Skellysolve" B-acetone (3:1) | U.V. | followed course of reaction | 225 |
| 10 | Phloroglucinol derivatives | see "Miscellaneous," No. 15 | | | | 380 |
| 11 | Phenolic aldehydes | sec "Aldehydes, etc.," No. 9 | | | | 396 |

TABLE 11.18—*Cont.*

| Compound | Absorbent | Developer | Visualization | Comments | Ref. |
|---|---|--|---|---|------------|
| 12 Phenolic aldehydes, ketones and acids | Silica Gel G | a. benzene-dioxane-HOAc (90:25:4) b. benzene-MeOH-HOAc (45:8:4) c. benzene-MeOH (95:5) | a. diazotized benzidine b. alkaline KMnO ₄ c. diazotized sulfanilic acid d. Rhodamine B | a. diagnostic b. quantitative | 288 290 |
| 13 Phenols and glycols | a. unbound aluminum oxide with HOAc | a. benzene | paper chromatography reagents | diagnostic | 144 |
| Nitro and amino phenols | b. unbound aluminum oxide | b. benzene-EtOH mixtures c. CHCl ₃ d. EtOEt | | | |
| 14 Phenols | Plaster-of-Paris-bound silica gel-oxalic acid | a. hexane-EtOAc (4:1)(3:2) b. benzene-ethyl ether (4:1) c. benzene | | diagnostic | 113 |
| 15 3,5-Dinitrobenzoates of alcohols and phenols | Silica Gel G | a. benzene-pet. ether (1:1) b. hexane-EtOAc (85:15)(75:25) c. toluene-EtOAc (90:10) | colored compounds | does not separate closely related compounds | 85 |
| 16 3,5-Dinitrobenzoates of alcohols | starch-bound silica gel-bentonite (4:1) with fluorescent material | hexane | U.V. | diagnostic | 209 |
| 17 3,5-Dinitrobenzoates of butyl and isoctyl alcohols | silica gel | | colored compounds | | 220 |
| 18 Phenols in pharmaceuticals | unbound acidic aluminum oxide | a. ether b. benzene-EtOH (95:5)(90:10) | a. KMnO ₄ -Fe(No ₃) ₃ b. KMnO ₄ | diagnostic | 340 |
| 19 Phenolic carboxylic acids | Kieselguhr G with Na ₂ WO ₄ , NaMoO ₄ or borax | a. organic layer of BuOH-HOAc-H ₂ O (4:1:5) b. butyl ether (sat. with H ₂ O)-HOAc (10:1) c. EtOAc-2-P-OH-H ₂ O (65:24:11) | Folin Denis Reagent | | 136 |

| | | | | | |
|----|----------|-----------------------------|--|--|--------------|
| 20 | Xylenols | Silica Gel G | a. benzene b. benzene-MeOH (95:5) | a. diazotized benzidine b. Echt blue salt | 301 |
| 21 | Alcohols | | | | 28 |
| 22 | Phenols | Silica Gel G and boric acid | EtOH-H ₂ O (90:30) containing 4% boric acid and 2% NaOAc as electrolyte | Diazotized benzidine | 291 |
| 23 | Phenols | ϵ -polycaprolactam | a. hexane b. cyclohexane c. benzene d. CHCl ₃ e. EtOAc | diazotized sulfanilic acid | 219a 436a |

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TABLE 11.19. PROTEINS, AMINO ACIDS AND THEIR DERIVATIVES

| Compound | Absorbent | Developer | Visualization | Comments | Ref. |
|--------------------------------------|---|--|-----------------------|---|-----------|
| 1 Amino acids | Silica Gel G | a. EtOH (96%)-H ₂ O (63:37) b. PrOH-H ₂ O (64:36) c. BuOH-HOAc-H ₂ O (60:20:20) d. phenol-H ₂ O (75:25) e. PrOH-NH ₄ OH (34% NH ₃) (67:33) f. EtOH (96%)-NH ₄ OH (34% NH ₃) (77:23) | modified ninhydrin | a. diagnostic b. two dimensional | 44 277 |
| 2 Amino acids | unbound aluminum oxide | a. H ₂ O b. pyridine-H ₂ O (1:1)(80:54) c. BuOH-EtOH-H ₂ O (60:40:40) | ninhydrin | a. diagnostic b. continuous elution c. spotted sample with NaOH | 267 |
| 3 Amino acids | buffered Silica Gel G (buffered with equal portions of 0.2 M KH ₂ PO ₄ and 0.2 M Na ₂ HPO ₄) | a. EtOH-H ₂ O (70:30) b. EtOH-NH ₄ OH (25% NH ₃) (4:1) c. EtOH-NH ₄ OH-H ₂ O (7:1:2) | paper chrom. reagents | diagnostic | 271 |
| 4 Amino acids | Silica Gel G | a. CH ₃ Cl-MeOH-NH ₄ OH (17% NH ₃) (2:2:1) in first direction b. phenol-H ₂ O (75:25 g/g) in second dimension c. methyl ethyl ketone-pyridine-H ₂ O-HOAc (70:15:15:2) for continuous | ninhydrin | a. diagnostic b. continuous development c. two dimensional | 99 |
| 5 Amino acids | Cellulose MN 300 Powder | a. upper phase of BuOH-HOAc-H ₂ O (4:1:5) b. pyridine-methyl ethyl ketone-H ₂ O (15:70:15) c. MeOH-H ₂ O-pyridine (80:20:4) d. six others | ninhydrin | diagnostic | 430 |
| 6 Amino acids | Silica Gel G | a. PrOH-H ₂ O (1:1) in first direction b. phenol-H ₂ O (10:4) in second direction | ninhydrin | a. diagnostic b. two dimensional | 279 |
| 7 Amino acids and similar substances | a. Silica Gel G b. Kieselguhr G c. Aluminum Oxide G | a. 440V, 19.6 m. amp. in 0.1 M sodium citrate buffer, pH 3.8 b. 460V, 12.6 m. amp. in 2 N HOAc-0.6 N formic acid (1:1) at pH 2 c. chromatography using BuOH-HOAc-H ₂ O (3:1:1) | ninhydrin | a. diagnostic b. electrophoresis c. combined electrophoresis and chromatog. | 136 |

| | | | | | | |
|----|--|--|---|--|---|-----|
| 8 | N-(2,4-Dinitrophenyl)amino acids | a. Silica Gel G b. Silica Gel G with phosphor | a. toluene-pyridine-ethylenechlorohydrin-0.8 NH ₄ OH (100:30:60:60), upper phase, in first direction. b. CHCl ₃ -benzyl alcohol-HOAc (70:30:3) in second direction c. Sol. a in one direction and CHCl ₃ -MeOH-HOAc (95:5:1) in the second direction as continuous chrom. d. Sol. a in one direction and benzene-pyridine-HOAc (30:20:2) in second direction as continuous chrom. e. CHCl ₃ - <i>t</i> -amyl alcohol-HOAc (70:30:3) | <i>N</i> a. colored compounds b. transmitted U.V. c. U.V. | a. Layers are all equilibrated with lower phase of sol. a before use b. continuous development c. two dimensional | 45 |
| 9 | Cit-auged N-(2,4-dinitrophenyl)-amino acids | Silica Gel G | a. toluene-pyridine-ethylenechlorohydrin-0.8 NH ₄ OH (100:30:60:60), upper phase in first direction b. CHCl ₃ -benzylalcohol-glac. HOAc (70:30:3) in second direction | radiographic Cl ₂ -toluidine | a. two dimensional b. layer equilibrated with lower phase of sol. a before use | 89 |
| 10 | 2-Phenyl-2-thiobutyanoins of amino acids | Silica Gel G | a. CHCl ₃ b. CHCl ₃ -MeOH (9:1) c. CHCl ₃ -formic acid (100:5) d. CHCl ₃ -MeOH-formic acid (70:30:2) e. Methyl ethyl ketone-pyridine-H ₂ O-conc. HOAc (70:15:15:2) | Cl ₂ -toluidine | diagnostic | 46 |
| 11 | Carbobenzoxy derivatives of amino acids and small peptides | Silica Gel G | a. BuOH-acetone-glac. HOAc-NH ₄ OH (7% NH ₃)-H ₂ O (4:5:1.5:1:1:2) b. BuOH-glac. HOAc-NH ₄ OH (7% NH ₃) (5:5:3:1:5) c. BuOH-HOAc-NH ₄ OH (7% NH ₃)-H ₂ O (6:1:1:2) d. BuOH-glac. HOAc-H ₂ O-pyridine (15:3:12:10) e. in case of two-phase systems, both were used, but layer dipped only in upper phase | a. ninhydrin b. K ₂ Cr ₂ O ₇ in conc. H ₂ SO ₄ | diagnostic | 95 |
| 12 | Amino acid and peptide derivatives | Silica Gel G | CHCl ₃ -MeOH (9:1) | Folin reagent | a. diagnostic b. monitored a synthesis | 163 |
| 13 | Polypeptides (up to five units) and derivatives | Silica Gel G | a. CHCl ₃ -acetone (9:1)(8:2) b. cyclohexane-EtOAc (1:1) c. CHCl ₃ -MeOH (9:1) | morin | diagnostic | 341 |

TABLE II.19—*Cont.*

| Compound | Absorbent | Developer | Visualization | Comments | Ref. |
|---|----------------------------------|--|---|---|------|
| 14 Polypeptides (up to ten units) and derivatives | Silica Gel G | a. BuOH sat. with 0.1% NH ₄ OH b. other systems used in paper chrom. | ninhydrin | a. diagnostic b. monitored a synthesis | 424 |
| 15 3-phenyl-2-thiohydantoin derivatives of di- and tripeptides | Silica Gel G | neptane-pyridine-EtOAc (5:3:2) | starch-iodine azide | diagnostic | 54 |
| 16 Proteins as such | polyamide-bound hydroxyl-apatite | potassium phosphate buffers, pH 6.5 of various concentrations | ninhydrin | a. diagnostic b. gradient elution | 153 |
| 17 Proteins and poly-peptides | Sephadex G 25 (moist) | a. 0.05 M NH ₄ OH b. H ₂ O | a. Pauli's reagent | a. diagnostic b. determine mol. wt. | 82 |
| Enzymes | "DEAE-Sephadex" A 25 | c. gradient elution involving addition of 0.02 M phosphate buffer pH 7.2 (which is also 1 M in NaCl) to the pure buffer | b. DPNH-pyruvic acid | c. gradient elution d. preparative | |
| 18 Carbobenzoxyaminocarbonyl derivatives of cyclic alcohols | Silica Gel G | CHCl ₃ -acetone (85:15) | Cl ₂ followed by KI and starch | a. monitored course of reaction b. monitored column chrom. | 456 |
| 19 Metabolic products of tryptophane | Silica Gel G | a. CHCl ₃ -MeOH-HOAc (75:20:5) b. methyl acetate-2-PrOH-NH ₄ OH (25% NH ₃) (45:35:20) | Ehrlich reagent | a. diagnostic b. urine analysis | 87 |

TABLE 11.20. STEROIDS

| | Compound | Adsorbent | Developer | Visualization | Comments | Ref. |
|---|--------------------------------------|--|--|--|---|------|
| 1 | Seventeen misc. steroids | Silica Gel G | a. benzene b. benzene-EtOAc (9:1)(2:1) c. cyclohexane-EtOAc (9:1)(19:1) d. 1,2-dichloroethane e. seven more | SbCl ₃ in CHCl ₃ | diagnostic | 416 |
| 2 | Seventy-nine steroids and sapogenins | unbound aluminum oxide, sometimes impregnated with morin | a. hexane-benzene mixtures b. benzene-EtOEt mixtures c. EtOEt-EtOH mixtures | a. conc. H ₂ SO ₄ b. U.V. c. morin spray | a. diagnostic b. preparative | 52 |
| 3 | Twenty-two misc. steroids | a. starch-bound silic acid b. Silica Gel G | EtOAc-cyclohexane mixtures | a. SbCl ₃ in CHCl ₃ b. 2,4-DNPH c. phosphomolybdic acid | diagnostic | 13 |
| 4 | Twenty-seven steroid estrogens | Silica Gel G | a. cyclohexane-EtOAc-EtOH (45:45:10) b. EtOAc-cyclohexane-EtOH (80:15:5) c. EtOAc-cyclohexane (50:50) d. CHCl ₃ -EtOH (90:10) e. EtOAc-cyclohexane-EtOH-HOAc (72:15:5:4:5:10) f. BuOH sat. with H ₂ O | a. Folin-Ciocalteau reag. b. Boure reagent c. 2% H ₂ SO ₄ in H ₂ O-EtOH (1:1) | a. diagnostic b. classification system | 221 |
| 5 | Forty-nine misc. steroids | unbound aluminum oxide | a. benzene-pet. ether mixtures b. benzene-EtOH mixtures | a. SbCl ₃ in CHCl ₃ b. SbCl ₃ in CHCl ₃ with 10% SOCl ₂ | diagnostic | 145 |
| 6 | Twenty-seven misc. steroids | a. unbound aluminum oxide | a. pet. ether-benzene mixtures b. benzene c. benzene-EtOH mixtures | reagents used in paper chromatography | diagnostic | 144 |
| | Steroid acids | b. unbound aluminum oxide-HOAc | d. benzene-EtOH (90:10) | | | |

Thin-Layer Chromatography

TABLE 11.20—*Cont.*

| Compound | Adsorbent | Developer | Visualization | Comments | Ref. |
|---|---|---|--|----------------------------------|------|
| 7 Steroids from fermentation reactions | Silica Gel G | CHCl ₃ -acetone mixtures | a. vanillin in H ₃ PO ₄ b. anisaldehyde in H ₃ SO ₄ c. SbCl ₃ in CHCl ₃ d. H ₃ PO ₄ e. 2,3,5-triphenyltetrazolium chloride f. HClO ₄ | diagnostic | 247 |
| 8 Thirteen misc. steroids | a. Silica Gel G with phosphor b. Aluminum Oxide G with phosphor | a. CHCl ₃ -EtOAc (3:2)(3:1) b. benzene-EtOAc (1:1)(9:1) c. hexane-EtOAc (1:1) | I ₂ vapor | a. quantitative b. diagnostic | 244 |
| 9 Fifty-five misc. steroids | a. starch-bound silica gel b. starch-bound silica gel-NaOH | a. hexane-EtOAc (4:1)(1:1) b. EtOAc c. benzene-2-PrOH (4:1) | a. phosphomolybdic acid b. many others | diagnostic | 363 |
| 10 Nineteen misc. steroids | plaster-of-Paris-bound “Celite” impregnated, by spraying, with a. formamide b. propylene glycol c. paraffin oil | a. hexane b. hexane-benzene (1:1) c. benzene d. CHCl ₃ e. ligroin f. MeOH-H ₂ O (95:5) | a. H ₃ PO ₄ b. tetrazolium blue c. isonicotinic acid hydrazide | a. diagnostic b. distribution | 414 |
| 11 Fifteen sterols and sterol derivatives | a. Silica Gel G b. Silica Gel G Kieselgur G (1:1) | a. cyclohexane-EtOAc-H ₂ O (600:400:1) (1560:440:1) b. isoctane-CCl ₄ (19:1) c. cyclohexane-heptane (1:1) | 50% H ₂ SO ₄ | diagnostic | 27 |

| 295 | | | | | |
|-----|-----------------------------|---|---|---|--|
| 12 | Sterols and sterol acetates | a. Kieselguhr G b. Kieselguhr G impregnated with undecane | a. cyclohexane-EtOAc (9:5:0.5) b. HOAc-H ₂ O (92:8)(90:10) c. HOAc-CH ₂ CN (1:3) | phosphomolybdic acid | diagnostic |
| 26 | | a. Silica Gel G b. Kieselguhr G c. a and b (1:1) d. all equilibrated with water by placing moist filter paper in the chamber | a. CH ₂ Cl ₂ -MeOH-formamide (93:6:1) b. toluene-Et ₂ O-formic acid (57:40:3) c. cyclohexane-acetone (1:1) d. cyclohexane-EtOAc-H ₂ O (600:400:1) e. CH ₂ Cl ₂ -MeOH-H ₂ O (485:15:1) f. three others | a. diagnostic b. distribution chrom. | |
| 405 | | Sllica Gel G | a. EtOAc b. butyl acetate | chlorosulfonic acid-HOAc (1:2) | diagnostic |
| 67 | | Sllica Gel G | a. benzene b. CHCl ₃ c. CHCl ₃ -MeOH (10:1)(97:3) | SbCl ₃ in CHCl ₃ | diagnostic |
| 68 | | Sllica Gel G | EtOAc-cyclohexane mixtures | SbCl ₃ in CHCl ₃ | diagnostic |
| 125 | | Sllica Gel G | benzene-EtOH (9:1) | SbCl ₅ in CCl ₄ | diagnostic |
| 394 | | Sllica Gel G | benzene-EtOH (9:1) | SbCl ₅ in CHCl ₃ | a. diagnostic b. quantitative |
| 391 | | unbound aluminum oxide | benzene-EtOAc mixtures | 70% H ₃ PO ₄ | a. diagnostic b. quantitative |
| 15 | | plaster-of-Paris-bound silicic acid | pentane-EtOAc mixtures | SbCl ₅ in CHCl ₃ | diagnostic |
| 347 | | a. aluminum oxide b. Silica Gel Reidel de Haen | a. benzene-CH ₂ Cl ₂ (2:1) b. cyclohexane-CH ₂ Cl ₂ (1:2) | I ₂ in KI | diagnostic |
| 408 | | Sllica Gel G | a. isopropyl ether b. isopropyl ether-EtOAc (5:2) c. benzene | chlorosulfonic acid-HOAc (1:2) | a. diagnostic b. preparative c. monitored course of reaction |
| | 21 | Misc. derivatives of pregnanol, cholesterol and sitosterol | | | |

Thin-Layer Chromatography

TABLE 11.20—Cont.

| Compound | Adsorbent | Developer | Visualization | Comments | Ref. |
|---|---|---|---|---|------------|
| 22 Pregnadiol | Silica Gel G | CHCl ₃ -acetone (9:1) | <i>ortho</i> -H ₃ P _O ₄ -phosphomolybdc acid | early test for pregnancy | 433 435 |
| 23 Cholesterol and its laurate ester | Silica Gel G | benzene-MeOH mixtures | conc. H ₂ SO ₄ | quantitative | 315 |
| 24 Cholesterol | Silica Gel G | hexane-EtOEt-HOAc (50:50:2) | HClO ₄ | a. quantitative b. microscope slides | 296 |
| 25 Blood cholesterol and cholesterol esters | Silica Gel G | a. benzene b. benzene-EtOAc (9:1) c. 1,2-dichloroethane d. CHCl ₃ e. two others | SbCl ₃ in CHCl ₃ | a. diagnostic b. semiquantitative | 415 |
| 26 Cholesterol esters | Silica Gel G | isopropyl ether-pet. ether (1:99) | SbCl ₃ in CHCl ₃ | a. diagnostic b. quantitative | 460 |
| 27 Cholesterol esters | Silica Gel G | benzene-pet. ether (40:60) | 50% H ₂ SO ₄ | diagnostic | 228 |
| 28 Cholesterol esters | plaster-of-Paris-bound silicic acid impregnated with paraffin | HOAc | a. I ₂ vapor b. SbCl ₃ in CHCl ₃ c. phosphonolybdc acid d. phosphotungstic acid | a. diagnostic b. reversed phase | 249 |
| 29 Bile acids, free and conjugated | Silica Gel G | a. HOAc-C ₂ H ₅ -isopropyl ether-isooamyl acetate-PtOH-benzenc (5:20:30:40: 10:10) for free acids b. propanoic acid-isooamyl acetate-H ₂ O-PtOH (15:20:5:10) for conjugate acids | phosphomolybdc acid | diagnostic | 154 |

| | | | | | | |
|----|---|------------------------------|--|--|---------------------------------------|-----|
| 30 | Cholic acid derivatives | Silica Gel G | a. toluene-HOAc-H ₂ O (5:5:1) upper phase b. BuOH-HOAc-H ₂ O (10:1:1) c. toluene-HOAc-H ₂ O (10:10:1) | a. phosphomolybdic acid b. H ₂ O | a. diagnostic b. quantitative | 115 |
| 31 | Adrenocorticotrophic hormones | Silica Gel G | EtOAc-sylyene-MeOH (90:5:5) | terrazolium chloride | diagnostic | 30 |
| 32 | Corticoids from urine | unbound silica gel | CHCl ₃ -EtOH (95:5) | terrazolium blue | diagnostic | 1 |
| 33 | Corticosteroids | Silica Gel G (not activated) | CHCl ₃ -MeOH-H ₂ O (485:15:1) (183:12:1)(90:10:1) | 50% H ₂ SO ₄ | diagnostic | 25 |
| 34 | Corticosteroids | plaster of Paris | a. acetone-benzene (1:4) b. EtOH-CHCl ₃ (3:97) | alkaline tetrazolium blue | diagnostic | 243 |
| 35 | Cholestanate derivatives | Silica Gel G | pet. ether-benzene (9:1) | I ₂ | diagnostic | 361 |
| 36 | Cholestanol derivatives | Silica Gel G | a. benzene b. benzene-Et ₂ OEt (70:30) c. pentane-EtOEt-benzene (20:20:60) | H ₂ O | quantitative | 125 |
| 37 | Aglycones of olean- der glycosides | Silica Gel G | EtOAc-CHCl ₃ (9:1) | SbCl ₃ in CHCl ₃ | diagnostic | 126 |
| 38 | Cardinolides | Silica Gel G (not activated) | benzene-EtOH (3:1) sat. with H ₂ O | a. trichloroacetic acid-chloramine-T b. SbCl ₃ -acetic anhydride | a. diagnostic b. preparative | 322 |
| 39 | Steroidal compounds involved in tomatin formation | Silica Gel G | EtOAc-cyclohexane (1:1)(3:7) | a. SbCl ₃ in chloroform b. 15% H ₃ PO ₄ | diagnostic | 337 |
| 40 | Digitoxigenin and derivatives | Silica Gel G | CHCl ₃ -EtOAc (1:1) | trichloroacetic acid-chloramine T | predicted solvents for column chrom. | 339 |
| 41 | Bile acids (free, conjugated) and derivatives | Silica Gel G | a. isooamyl acetate-C ₂ H ₅ -isopropyl ether-benzene-PrOH-H ₂ OAc (4:3:2:1:0.5) b. isooamyl acetate-propanoic acid-P ₂ O ₅ -H ₂ O (4:3:2:1) c. cyclohexane-EtOAc (6:4) | phosphomolybdic acid | a. diagnostic b. microscope slides | 90 |
| | | | | | | 152 |

Thin-Layer Chromatography

TABLE 11.20—Cont.

| | Compound | Absorbent | Developer | Visualization | Comments | Ref. |
|----|--|---|---|--|---|------|
| 42 | Steroids from bull testicles | a. plaster-of-Paris-bound silica gel b. EtOAc c. butyl acetate d. several others | a. benzene-EtOAc (8:2) b. EtOAc c. butyl acetate d. several others | conc. H ₂ SO ₄ -U.V. | diagnostic | 272 |
| 43 | Radioactive androstanic acid derivatives | Silica Gel G | cyclohexane-EtOAc (3:2) | radiographic | a. diagnostic b. quantitative c. preparative | 349 |
| 44 | β -Sitossterol from potato beetles | Silica Gel Riedel dc Haen | pet. ether-EtOAc-benzene (85:10:5) | SbCl ₃ in HOAc | diagnostic | 348 |
| 45 | 1,3-Cholestadiene methyl ether | Silica Gel G | benzene-EtOEt (4:1) | I ₂ vapor | monitored column chrom. | 397 |
| 46 | Aldosterones from urine | Silica Gel G | a. cyclohexane-2-PROH (7:3) b. CHCl ₃ glac. HOAc (8:2) | tetrazolium blue | a. two dimensional b. developed in dark c. quantitative | 278 |
| 47 | Saponin from <i>Solanum</i> species | Kieselguhr G | EtOAc-cyclohexane (7:3) | vanillin in H ₃ PO ₄ | diagnostic | 336 |

TABLE 11.21. TERPENOIDS

| | Compound | Adsorbent | Developer | Visualization | Comments | Ref. |
|---|------------------------------------|--|---|--|---|------------|
| 1 | Forty-nine misc. terpenoids | starch-bound silicic acid | hexane | a. fluorescein-Br ₂ b. H ₂ SO ₄ -HNO ₃ | a. diagnostic b. monitored column | 190 |
| 2 | Fourteen misc. terpenoid | starch-bound silicic acid with phosphors | hexane-EtOAc (85:15) | a. fluorescein-Br ₂ b. U.V. c. σ -dianisidine d. conc. H ₂ SO ₄ e. bromoresol green f. H ₂ SO ₄ -HNO ₃ | a. diagnostic b. predicted solvents for column chrom. c. monitored column | 193 251 |
| 3 | Twenty-eight oxygenated terpenoids | starch-bound silicic acid | a. hexane-EtOAc (85:15) (10:30) b. CHCl ₃ (alcohol-free) EtOAc (90:10) (95:5) c. 1-nitropropane-hexane (50:50) d. ethyl carbonate-CHCl ₃ (15:85) e. hexane-isopropyl formate (70:30) f. four others | a. fluorescein-Br ₂ b. σ -dianisidine c. H ₂ SO ₄ -HNO ₃ d. conc. H ₂ SO ₄ | a. diagnostic b. chemical reactions on layers | 252 |
| 4 | Twelve misc. terpenoids | unbound alumina | a. pet. ether-benzene mixtures b. benzene c. benzene-EtOH mixtures | resents used for paper chromatography | diagnostic | 144 |
| 5 | Diterpenoids | silicic acid | hexane-EtOAc (4:1) | KMnO ₄ | a. diagnostic b. monitored column c. review | 213 |
| 6 | Eighteen triterpenoids | synthetic fibrous alumina | a. hexane-benzene (8:1) b. cyclohexane-EtOAc (85:15) c. benzene d. EtOEt e. EtOEt-EtOH (98:2) | a. 2,4-DNPH b. SbCl ₅ in CCl ₄ c. Rhodamine B | diagnostic | 165 |

Thin-Layer Chromatography

TABLE II.21—*Cont.*

| | Compound | Adsorbent | Developer | Visualization | Comments | Ref. |
|----|--|--|--|---------------------------------------|---------------------------------|------------|
| 7 | Sixteen triterpenoids | Silica Gel G | a. isopropyl ether-acetone (5:2)(19:1) b. isopropyl ether c. cyclohexane d. benzene e. CH_2Cl_2 | chlorosulfonic acid- HOAc | diagnostic | 406 407 |
| | Twenty-three triterpene acids | — | f. sol. a g. sol. a plus 5% pyridine h. EtOAc-MeOH -diethylamine (14:4:3) i. chlorobenzene- HOAc (9:1) j. CH_2Cl_2 -pyridine (7:2) | — | — | — |
| 8 | Triterpene acids | Silica Gel G | EtO_2I -hexane (1:1) | 50% H_2SO_4 | diagnostic | 421 |
| 9 | Triterpene acids from <i>Cammidiaria glandulosa</i> Schinz, as methyl esters | — | $\text{CHCl}_3\text{-EtOAc}$ (4:1) | conc. H_2SO_4 | diagnostic | 402 |
| 10 | Carotenoids | a. Silica Gel G- $\text{Ca}(\text{OH})_2$ (1:4) b. Silica Gel G- $\text{Ca}(\text{OH})_2$ (1:4) impregnated with paraffin | a. benzene-pet. ether (1:1) b. sol. a with 1% MeOH c. MeOH sat. with paraffin | colored compounds | a. diagnostic b. preparative | 446 449 |
| 11 | Isomeric methols | Silica Gel G | a. benzene b. MeOH c. benzene- MeOH (95:5)(75:25) | SbCl_5 in CCl_4 | diagnostic | 299 |
| 12 | Ipomeamarone | starch-bound silica gel | hexane- EtOAc (9:1) | 2,4-DNPH | quantitative | 2,76 |

| | | | | | | |
|----|-------------------------------|---|--------------------------|-----------------|------------|-----|
| 13 | Isophytol and geranyl-linalol | starch-bound silica gel | hexane-EtOAc (85:15) | | diagnostic | 81 |
| 14 | Azulene and cadalene | a. starch-bound silica gel b. starch-bound alumina | a. pet. ether b. EtOH | trinitrobenzene | diagnostic | 108 |
| 15 | Terpenoids of mint oils | see "Essential Oils," No. 11 | | | | 20 |

Thin-Layer Chromatography

TABLE 11.22. VITAMINS

| | Compound | Adsorbent | Developer | Visualization | Comments | Ref. |
|---|---|---|--|---|--|------------|
| 1 | Carotenes and fat-soluble vitamins A, D, E, K | unbound aluminum oxide | a. MeOH b. CCl ₄ c. xylene d. eleven others | a. 70% HClO ₄ b. conc. H ₂ SO ₄ | diagnostic | 70 |
| 2 | Carotenes and Vitamin A | unbound aluminum oxide | pet. ether | a. SbCl ₃ in CHCl ₃ b. colored compounds | a. diagnostic b. quantitative | 210 211 |
| 3 | B vitamins | Silica Gel G with fluorescent material | HOAc-acetone-MeOH-benzene (5:5:20:70) | a. U.V. b. potassium iodoplatinum dichloroquinone-chlorimide-NH ₃ c. ninhydrin | a. diagnostic b. carried out in dark | 116 |
| 4 | Cyanocobalamin and hydroxycobalamin | Silica Gel G | a. MeOH b. PrOH c. BuOH-HOAc-H ₂ O (20:5:1) (20:5:5) and others d. BuOH-HOAc-MeOH (20:10: 25) e. sixteen others | a. microbiologically b. spectrophotometrically | a. diagnostic b. quantitative | 55 |
| 5 | Vitamins K ₂₍₂₀₎ , K ₂₍₁₀₎ and K ₂₍₁₀₎ | Silica Gel G with sodium fluorescein | a. heptane-benzene (1:1) dried and followed by benzene | U.V. | a. diagnostic b. radioactive c. quantitative | 35 |
| 6 | Tocopherol mixtures | a. Silica Gel G b. Aluminum Oxide D5 | a. CHCl ₃ b. benzene | a. ceric sulfate b. phosphomolybdic acid-NH ₃ | a. diagnostic b. quantitative | 354 |

| | | | | | |
|----|----------------------|---|--|---|------------|
| 7 | Retinen | a. Silica Gel G | a. Pet. ether-benzene mixtures b. MeOH sat. with paraffin | rhodanin | 448 |
| | | b. Silica Gel G impregnated with paraffin | | | |
| 8 | Fat-soluble vitamins | alumina | a. HClO ₄ -70% b. H ₂ SO ₄ -98% | 37 | 431 |
| | | | | | |
| 9 | Ubiquinones | a. Silica Gel G | a. Benzene-CHCl ₃ (1:1) b. acetone-paraffin (sat. with H ₂ O) (9:1) | a. Rhodamine B b. SbCl ₃ in CHCl ₃ | diagnostic |
| | | b. Silica Gel G impregnated with paraffin | | | |
| 10 | Vitamins | | | | 280 |

TABLE 11.23. MISCELLANEOUS

| Compound | Adsorbent | Developer | Visualization | Comments | Ref. |
|--|--|---|--|--|------------|
| 1 Benzquinones from <i>Myriapodes</i> | starch-bound silicic acid | hexane-EtOAc (83:15) | | a. diazotized diagnostic b. phosphomolybdic acid | 12 |
| 2 Antioxidants (phenolic) | unbound polyamide powder | MeOH-acetone mixtures | a. diazotized sulfanilic acid b. phosphomolybdic acid | a. monitored column b. diagnostic | 72 |
| 3 Anioxidants, synthetic | Silica Gel G | a. benzene b. CHCl ₃ c. CHCl ₃ -methyl cellosolve (80:20) | a. phosphomolybdic acid b. diazotized sulfanilic acid c. 2,6-dichloroquinonemchlorimide, neutral and buffered | a. diagnostic b. two dimensional c. quantitative | 352 |
| 4 Alkyl phosphenosides, sulfides, sulfones, and sulfoxides | Silica Gel G | a. acetone b. CHCl ₃ | a. Na ₂ Cr ₂ O ₇ -H ₂ SO ₄ b. KMnO ₄ -H ₂ SO ₄ c. FeCl ₃ and K ₃ Fe(CN) ₆ | diagnostic | 98 |
| 5 Thiophene derivatives | a. Silica Gel G b. Aluminum Oxide G | a. benzene-CHCl ₃ (9:1) b. MeOH c. pet. ether | a. U.V. b. isatin in H ₂ SO ₄ | diagnostic | 65 |
| 6 Aromatic amines and nitrocompounds | see "Amines," No. 5 | | | | 110 |
| 7 Adulteration of the drug <i>Radix Zingiberiae</i> DAB6 | Silica Gel G | CHCl ₃ | SiO ₂ in CCl ₄ | diagnostic | 161 |
| 8 Diphenyl in citrus fruits | starch-bound silicic acid with phosphors | pet. ether | U.V. | quantitative | 194 389 |
| 9 Polycyclic aromatics | acetylated cellulose powder | MeOH-EtOEt-H ₂ O (4:4:1) | fluorescein | diagnostic | 82 |
| 10 Nitramine explosives | Silica Gel G | pet. ether-acetone (2:102) | diphenylamine | diagnostic | 216 |

| | | | | | | |
|----|--|--|---|--|--|-----|
| 11 | Peroxides | starch-bound silica gel | CCl ₄ -acetone (2:1) | a. KI b. ferrous ammonium sulfide and potassium thiocyanate | diagnostic | 242 |
| 12 | Piperidoles, quinolones and cyclohexanedi-ones | unbound aluminum oxide | a. benzene-acetone (1:1) b. acetone-MeOH (3:1) | a. I ₂ b. U.V. | tested procedure | 254 |
| 13 | Honeybee larval foods | silicic acid | MeOH-CHCl ₃ (29:71) | a. p-anisidine b. ninhydrin c. FeCl ₃ d. I ₂ e. conc. H ₂ SO ₄ | diagnostic | 292 |
| 14 | Plasticizers (phthalates, phosphates and other esters) | Silica Gel G with fluorescent material | a. isoctane-EtOAc (90:10) b. benzene-EtOAc (95:5) c. butyl ether-hexane (80:20) | a. phosphomolybdic acid b. resorcinol-EtOH-ZnCl ₂ followed by H ₂ SO ₄ and KOH c. alcoholic KOH followed by urea d. 2,6-dichloroquinonemchlorimide followed by borax e. five others | diagnostic | 293 |
| 15 | Phloroglucinol-butanones from <i>Dryophytes</i> | a. Silica Gel G buffered with NaOAc b. Silica Gel G impregnated with paraffin | a. EtOAc b. MeOH-formic acid-H ₂ O (75:10:15) | Folin-Ciocalteau reagent-NH ₃ | diagnostic | 380 |
| 16 | Chamazulene derivatives and dyes as test substances | a. silicic acid b. starch-bound silicic acid | hexane-EtOAc (9:1) | | methods exploration | 381 |
| 17 | Uronic acid | Silica Gel G | a. benzene b. MeOH | a. U.V. b. FeCl ₃ | a. diagnostic b. on microscope slides | 437 |
| | Aminopyrine | | | | | |

TABLE 11.23—*Cont.*

| Compound | Adsorbent | Developer | Visualization | Comments | Ref. |
|--|--|---|---|----------------------------------|------|
| 18 Neuraminic acid derivatives | Silica Gel G | a. PrOH-1 N NH ₄ OAc-H ₂ O (6:2:1) b. PrOH-H ₂ O (7:3) | a. Bial's reagent b. thiobarbituric acid-HIO ₄ -ethylene glycol | diagnostic | 127 |
| 19 Narcotics in forensic chemistry | Silica Gel G with fluorescent material | piperidine-pet. ether (1:5) | U.V. | diagnostic in urine | 92 |
| 20 Lumiflavin derivatives | Silica Gel G | a. 2 N HClO ₄ -dimethylsulfoxide-BuOH (12:2:6) b. HOAc-H ₂ O-BuOH (2:2:6) | colored compounds | diagnostic | 143 |
| 21 Bufadienolides from toad poisons | Silica Gel G | a. EtOAc b. EtOAc-cyclohexane (80:20) c. EtOAc-acetone (90:10) d. EtOAc sat. with H ₂ O | SbCl ₃ in CHCl ₃ | diagnostic | 457 |
| 22 Adrenaline and its derivatives | Silica Gel G | CHCl ₃ -MeOH (9:1) | H ₄ PO ₄ -phosphomolybdic acid | a. diagnostic b. quantitative | 434 |
| 23 Soladulcidin, a nitrogen-containing aglycone from glycosides of <i>Solanum dulcamara</i> L. | Silica Gel G | EtOAc-cyclohexane (7:3) | vanillin-H ₂ PO ₄ | diagnostic | 338 |
| 24 Anthocyanins | polyacrylonitrile-polyamide (7:1) | 1-pentanol-PrOH-HOAc-H ₂ O (3:2:2:1) plus 1-2% 1-heptanol | colored compounds | a. diagnostic b. descending | 36 |
| 25 Olefins as mercury salts | Silica Gel G | PrOH-triethylamine-H ₂ O (50:25:25) | diphenylcarbazide | diagnostic | 309 |

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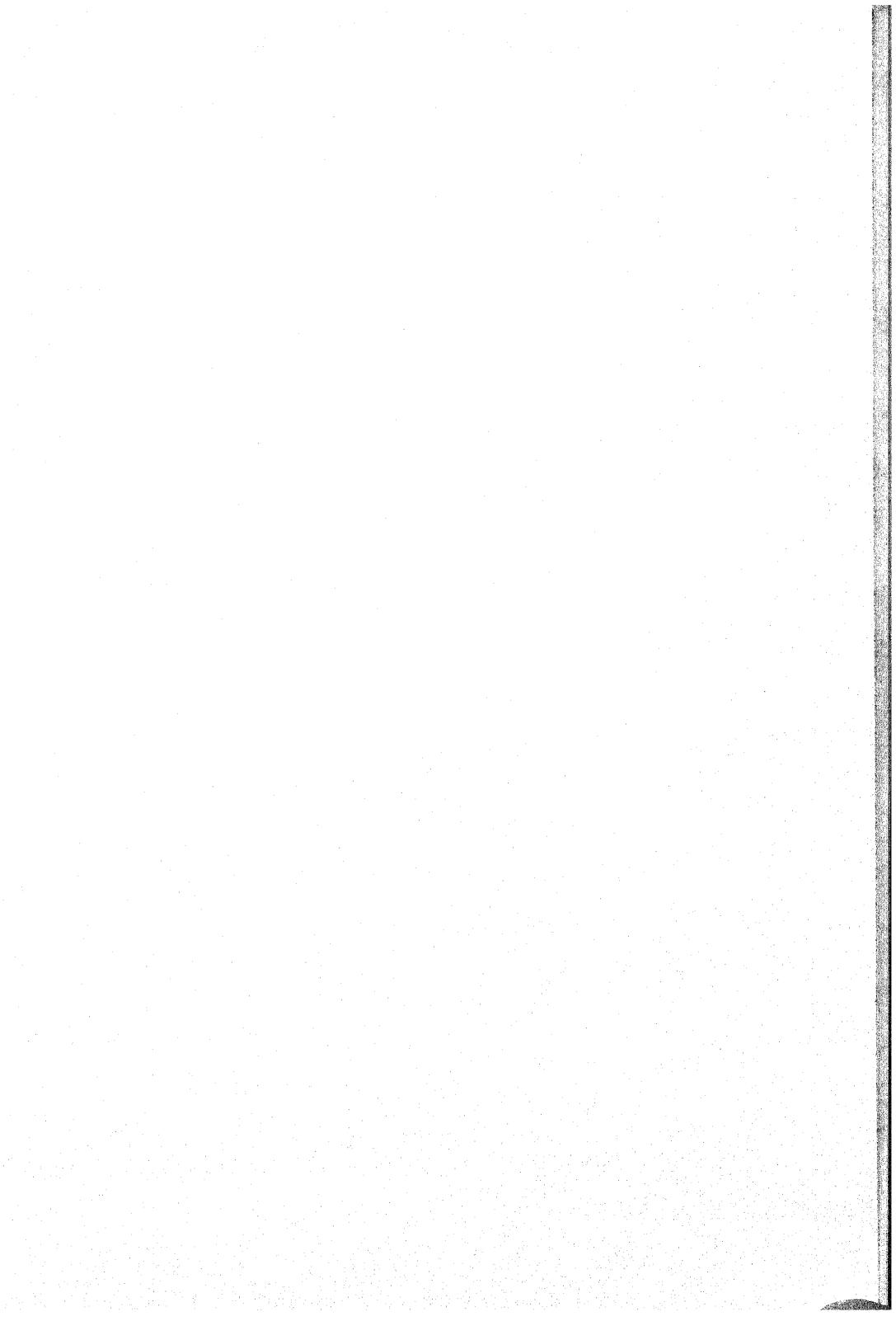
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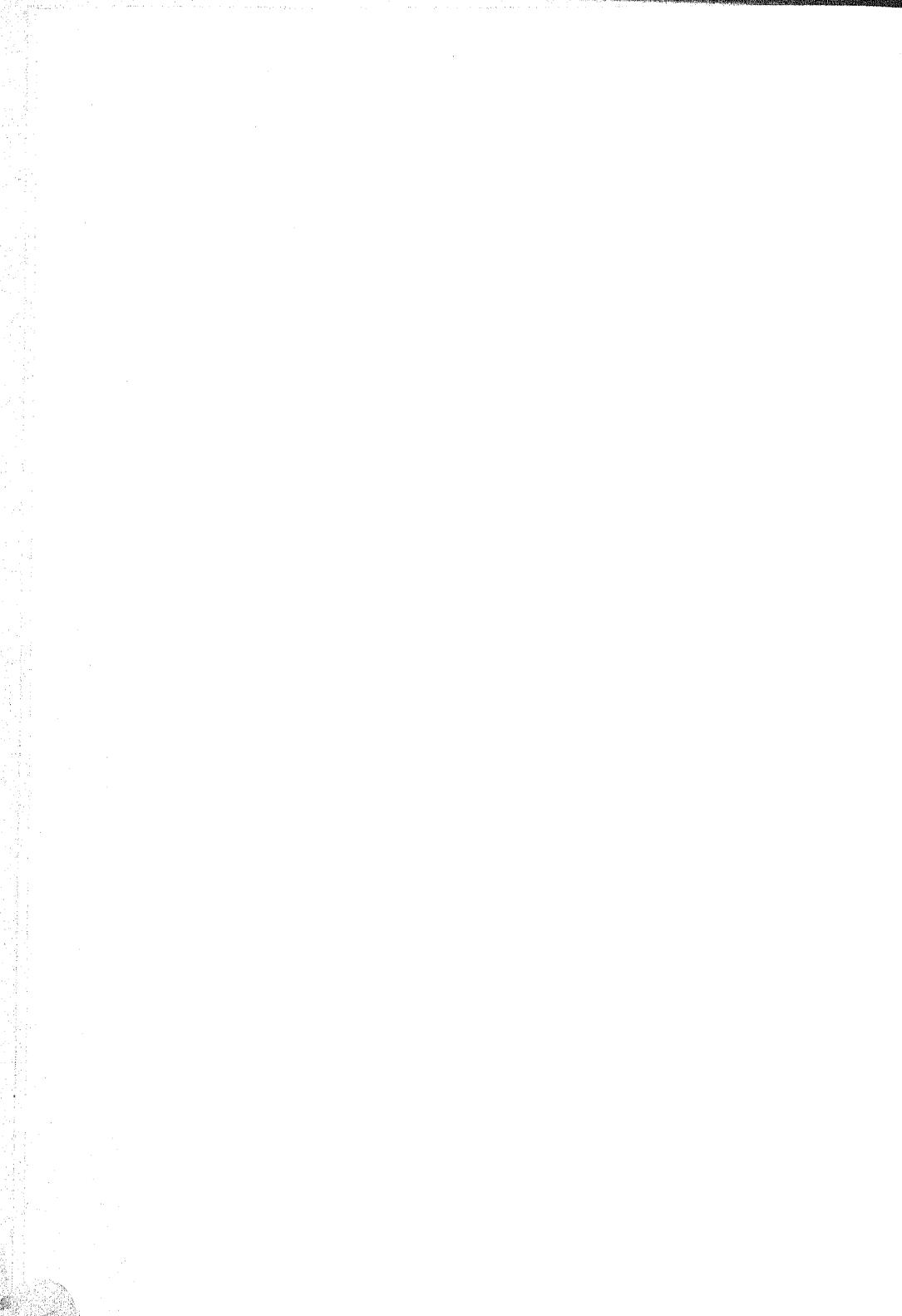
THIN-LAYER

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With a Foreword by
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Foreword

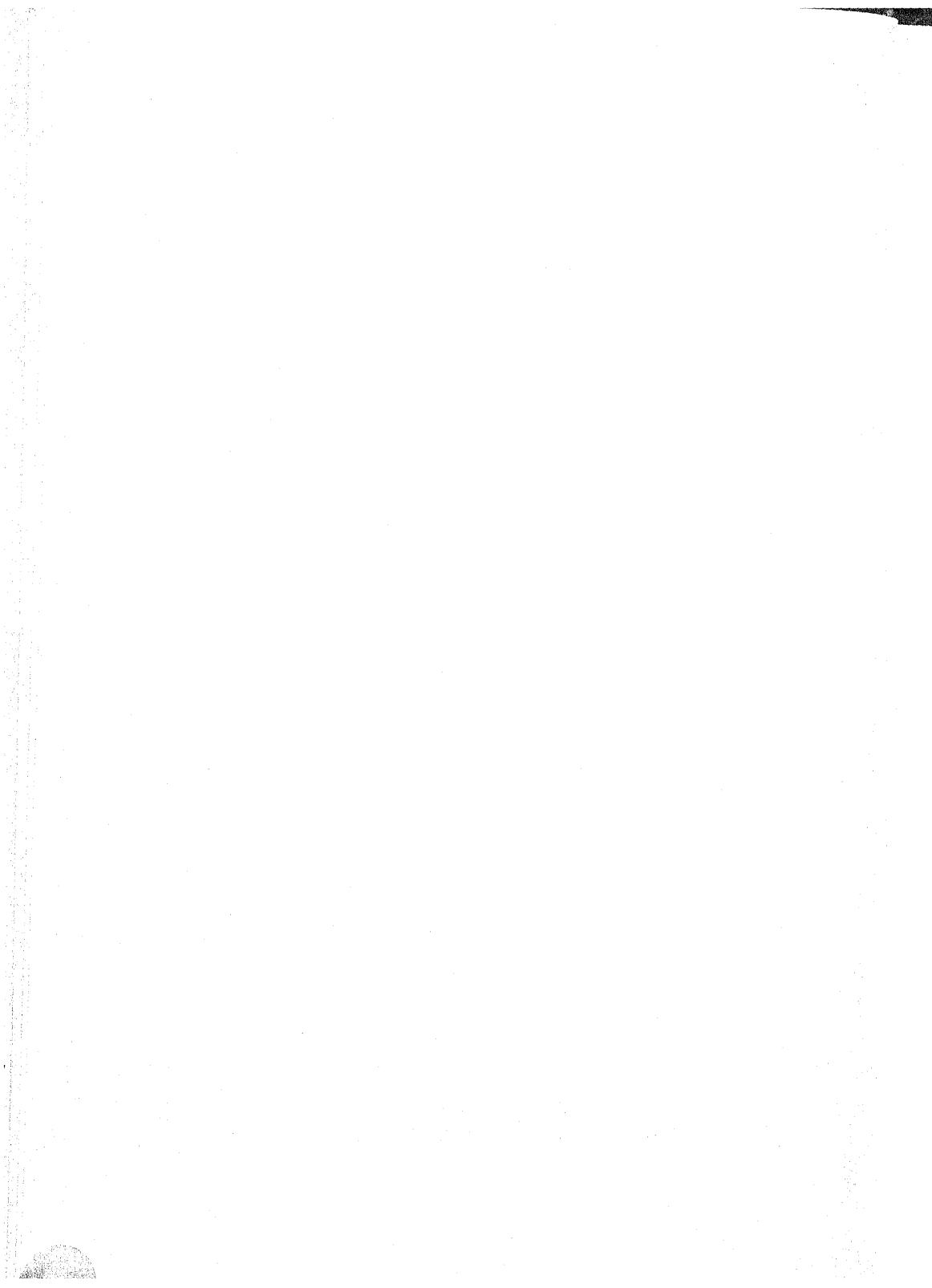
Thin-layer chromatography (as it is known today) was developed because of a specific need for a rapid method which would separate small amounts of compounds. Soon after its development it was apparent that the method was more than a micromethod for separating compounds. It was a means of (1) investigating absorbents and solvents from column work, (2) following the course of elution chromatography of colorless compounds, (3) checking the course of reactions and (4) carrying out certain reactions such as oxidations, reductions, dehydrations and so forth, directly on the strip or plate. By these means and other reactions applied directly to the unknowns, an insight into the type of compound could be gained. All these functions of thin-layer chromatography are enhanced by the speed of the method and the fact that only minute amounts of material are needed.

In general, a new technique that is developed for the separation and identification of compounds is not a method which, *per se*, displaces a previous technique. Rather it is an addition to the numerous tools available to the scientist for solving his problems.

A man that adopts the idea that a given technique is the solution to all his problems is missing an opportunity. Many times combinations of various techniques give results which surpass anything that can be attained with any of the individual techniques. Keeping this in mind it is to be hoped that this book will be an introduction to a method to those who have not used it and an aid to those who wish to further their knowledge of the subject.

J. G. KIRCHNER

Linden, N.J.



Preface

Chromatography on thin layers of adsorbent was conceived as early as 1938 and developed largely during the early 1950's. However, it has been used more extensively since about 1958 and now appears to be in an era of rapid development and almost universal adoption. At such a time, a book setting forth the basic technical details and surveying the field is in order. The author first observed the technique in the laboratory of Professor Hans Schmid in Zürich, Switzerland and was much impressed. This impression and the successful application of the method in his own laboratory have prompted the writing of this book.

The text and the bibliography cover the majority of the literature through December 1962 and a few particularly pertinent papers in 1963.

It is the intention of the author to write a condensed and non-theoretical description of this simple laboratory technique. Thus, the experimental aspects and "tricks" will be taken up, catalogued and discussed in considerable detail. In the last Chapter, an attempt will be made to tabulate, in a concise and meaningful way, as many specific applications of the method as possible. Any theoretical treatment of the subject must consist of two portions. The first is a discussion of general chromatographic theory and the second is a consideration of the deviations from the theory which arise in thin layers. Such a discussion is beyond the scope of this book.

The author would like to express his appreciation to the following persons who have made his career—and, thereby, this book—possible: Professors James L. Hall, C. L. Lazzell, L. A. Pursglove, Katherine Wilson and Elizabeth Frost Reed who are or were on the Staff of

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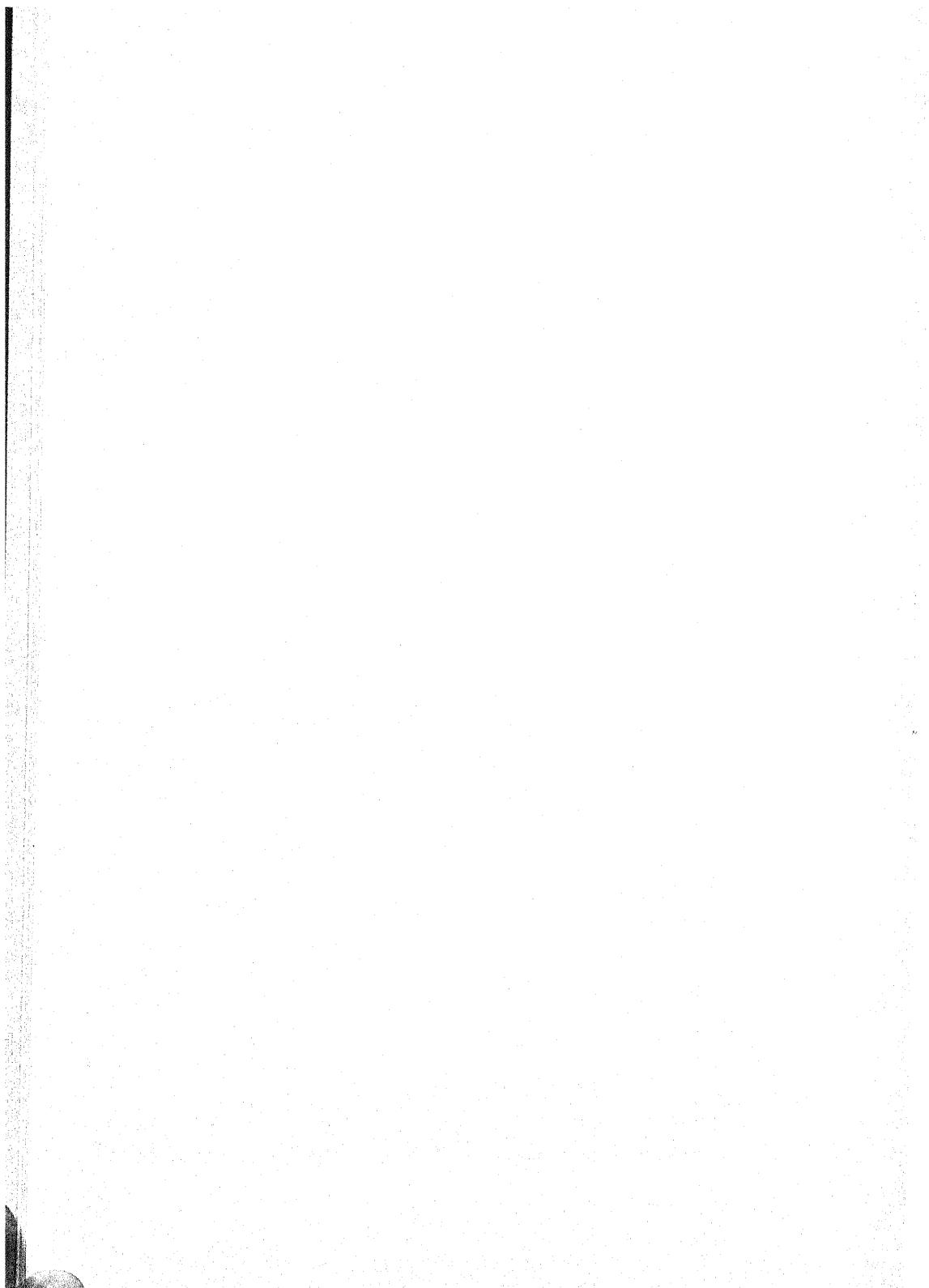
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CHAPTER 1

Introduction, History and General Applications

INTRODUCTION

Since the very beginning of the science of chemistry, its practitioners have been plagued by two technical problems. The first is to learn the purity of a given preparation or, conversely, how many components are in a given system. This information is necessary regardless of whether the system is a mineral mixture, an extract of some plant or animal, or the result of a chemical reaction. The second problem is to resolve the system into its pure components so that they can be characterized and studied. The earlier chemists performed phenomenal feats by fractional crystallization and distillation, but it has only been in the last fifty years or so that the various techniques of chromatography have promised true solutions to these problems.

It follows from the stated problems that chromatography can be considered from two viewpoints. One is *diagnostic** or qualitative and the second is *preparative.** The object of the former is to determine the number of components in a system and to learn, if possible, what they are without actually isolating them. The latter viewpoint involves the separation of a mixture into its components in such a way that reasonable amounts can be isolated and studied. Still a third aspect is *quantitative*, or concern about how much of each component is present.

* These concepts and others in this section have been well reviewed by Keulemans¹⁸⁷ and will be only briefly discussed in this volume.

All of the techniques of chromatography are based upon the same simple principle. They involve a moving system of some type (liquid or gas) which is in equilibrium with a stationary phase. These phases are so designed that the mixture to be separated will be distributed between the two. When the stationary phase is a solid and the forces acting between it and the mixture are adsorptive in nature, the technique is called *adsorption chromatography*. When the stationary phase is a simple liquid or a liquid held on some type of support, the chromatography is considered to be *partition chromatography*.

In general, adsorption chromatography involves a relatively non-polar moving phase and works best when the substances to be separated are not very polar. The major advantages over partition chromatography are that larger quantities can be separated in comparable systems and that a controlled temperature is not necessary. Partition chromatography, on the other hand, generally involves polar solvents and mixtures of very polar compounds such as carbohydrates or amino acids. Since it is basically dependent upon the distribution coefficients of the substances in question, which are, in turn, highly sensitive to temperature and other conditions, a carefully controlled atmosphere is required. The line of demarcation between adsorption and partition chromatography is, however, often blurred.

At this point, it would be appropriate to define some of the terms as they will be used throughout this book. The *adsorbent* will be the finely divided powder which makes up the stationary phase in adsorption chromatography, or holds the stationary liquid in partition chromatography. The *layer* will refer to a thin layer of absorbent, bound or unbound, deposited on a glass plate. The term *spotting* will refer to the application of the substance to be separated to the thin layer. The passing of a liquid through the layer to affect a separation will be referred to as *development* and the liquid itself will be consistently called the *developer*. The term *eluent* will be used only when the complete removal of a substance from the adsorbent is intended. The term *visualization* will refer to the rendering visible of the results of a developed chromatogram. The *Rf* will be the distance traveled by a given substance divided by the distance traveled by the solvent front. Both are measured from the origin.

Column chromatography, as originated by Tswett^{409,410} and since

practiced by legions of chemists, was primarily an adsorption technique used for preparative work. Column chromatography using an adsorbent such as silica gel or cellulose as a support for a liquid was conceived by Martin and Synge²⁴¹ and is partition chromatography for a preparative purpose. The countercurrent distribution of Craig and his co-workers^{59,60} may or may not be defined as chromatography but at least involves similar phenomena. It might also be considered a partition technique with a preparative goal. None of the above techniques have been particularly successful as diagnostic methods due to the extensive labor involved in the preparation of a single chromatogram.

The first major development in diagnostic chromatography was the paper chromatography pioneered by Consden, Gordon and Martin.^{57a,57b} The method was fantastically successful and was rapidly adopted all over the world and in every type of laboratory. The major advantages of paper chromatography are its extreme simplicity and the fact that relatively inexpensive equipment is needed. However, since it is a partition technique with the stationary liquid phase held on a piece of paper, it works best with polar developers and small amounts of polar substances. While techniques of preparative paper chromatography have been worked out and used successfully, they are laborious.

The second, primarily diagnostic technique of chromatography was gas chromatography. This was either an adsorption chromatography, as pioneered by Turner,⁴¹² Claesson⁵⁸ and Cremer,^{61,62} or a partition chromatography, as suggested by Martin and Synge²⁴¹ and introduced by James and Martin.¹⁷⁰ The advantages of gas chromatography are its speed of operation, its almost unbelievable degree of resolution and the fact that the results can be interpreted quantitatively. The disadvantages are that relatively expensive and complex equipment is needed and that the substances to be separated must have at least some vapor pressure at workable temperatures. The use of gas chromatography as a preparative technique has been quite successful although hampered by technical difficulties.

Thus, it would appear that a diagnostic technique which is adsorptive in nature and which combines the technical simplicity of paper chromatography and the speed of gas chromatography is lack-

ing. Ideally, such a method should lend itself to quantitative interpretation and should be useful also as a preparative method. Most of the conditions are fulfilled by *thin-layer chromatography*, or, as it is sometimes called, "thin-film," "open-column," "chromatostrip" or "chromatoplate" chromatography. In essence, this is a type of adsorption chromatography where the adsorbent is a thin layer of some solid deposited on a glass plate support. In operation, it is analogous to paper chromatography; that is, the substance to be separated is placed a short distance from one end of the layer and is resolved by a solvent passing through the layer by capillary action. The development is carried out in a simple closed system as in paper chromatography, but is much more rapid. When the proper solvent mixtures are used, the method can become a partition technique. Thus far, the quantitative interpretation has involved errors of 3 to 5 per cent, but the method shows promise as a preparative means for quantities of one gram or less.

HISTORY OF THIN-LAYER CHROMATOGRAPHY

It is always difficult to sort out the various originators of some concept or technique and to assign credit, because any such development is the result of contributions and ideas from many people and laboratories. Such contributions vary from the initial flash of genius to the more practical aspects such as the introduction of new or more convenient equipment or materials. The historical development of thin-layer chromatography is best divided into two such phases. The first phase is the conception and initial development of equipment and techniques followed by their slow but steady adoption. The second phase is an extensive and rapid development following the invention of new equipment, the standardization of the method and, particularly, the commercial availability of apparatus and adsorbents. A third phase will surely follow in which the method becomes a routine technique applied universally and without particular thought and attention.

In 1938, Izmailov and Shraiber¹⁶⁹ described the use of thin layers of adsorbent on glass plates for the separation of galenicals. The English summary of this paper is as follows:

"A method for chromatographic adsorption analysis is elaborated, based on the observation of the division of substances into zones on a thin layer of adsorbent, using one drop of the substance. The results obtained by the method proposed are qualitatively the same as those obtained by the usual chromatographic adsorption method of analysis. The method enables one to obtain satisfactory results using one drop of the substance under test, very small quantities of the adsorbent and minimal time. The method may be used for the evaluation of galenical preparations and their identification, as well as for a preliminary test of the adsorbent and the kind of developer."

In 1941, Crowe⁶³ described the use of thin layers of unbound adsorbent to help predict the best solvents for column chromatography. In a book entitled "Introduction to Chromatography"⁴⁴³ which was published in 1947, Williams reported the use of thin layers of adsorbent held between horizontal glass plates. The top plate had a small hole for application of the sample and the developer, and the chromatogram was made in a circular fashion.

The use of a binding agent (starch) to hold the layers in place was introduced by Meinhard and Hall²⁴⁶ in 1949. The resulting layers of alumina—"Celite" prepared on microscope slides, were used for the separation, again in a circular fashion, of inorganic salts.

The next and, by far, the most extensive and comprehensive contributions to the development of thin-layer chromatography were made by J. G. Kirchner, J. M. Miller and their co-workers at the U. S. Department of Agriculture Laboratories in Pasadena. In an extensive series of papers (1951-1957) this group investigated various adsorbents and binding agents,¹⁹³ designed equipment for the preparation of "chromatostrips"²⁵³ and used the technique for the investigation of the terpenoids.^{190-194,251-253} Narrow glass plates called "chromatostrips" (0.5 by 5.25 in.) with a silicic acid-starch layer were found to be most useful for their work although they also introduced the use of larger plates.¹⁹¹ The larger plates called "chromatoplates" by Reitsema³²³ were used by him in extensive work on essential oils.³²³⁻³²⁷

Starting a little later, but roughly paralleling the work of Kirchner and Miller, Mottier and Potterat²⁶⁸ worked out a method for analyzing food dyes on layers of nonbonded alumina and even separated amino acids by this technique.²⁶⁷ Some of the other workers in the

earlier phase (prior to 1956) were Ito, Wakamatsu and Kawahara,^{167,168} Labat and Montes,²⁰⁹ Grüner and Spaich,¹³¹ Coveney, Matthews and Pickering,⁵⁸ G. Wagner,⁴²⁶ Rigby and Bethune,³²⁸ Bryant,⁵¹ Lagoni and Wortmann,^{210,211} Demole,⁷⁶ Fukushi and Obata,¹⁰⁸ Onoe²⁸⁴ and Maruyama.²⁴² The earlier phases are well reviewed by Demole in French⁷⁷ and English.⁷⁸

The major reason for the slow development and adoption of the technique during the time from 1951 to 1958 was the fact that equipment and chemicals were not commercially available. More inertia must be overcome in the construction of equipment and the preparation of adsorbents than in signing a check or placing an order.

This last obstacle was removed by Egon Stahl in Germany. Stahl, working with C. Desaga, G. m. b. H., in Heidelberg, devised a system involving standard-size glass plates (5 by 20 cm and 20 by 20 cm), a new apparatus for the preparation of layers, and a standard adsorbent (silica gel-plaster of Paris). Subsequently, the apparatus became commercially available from Desaga and the adsorbent became available from E. Merck, A. G., in Darmstadt. Stahl and his co-workers studied the variables of the technique in a thorough manner,³⁸¹ applied it to many new types of organic compounds³⁶⁶⁻³⁸² and pioneered the use of new adsorbents. These new adsorbents immediately became commercially available. Stahl called the method "Dünnschicht-Chromatographie" or "thin-layer chromatography" ("TLC") and this name seems to be widely accepted.

The applications of the thin-layer technique mushroomed after 1958 so that it is impossible to mention all of the people who have contributed. However, some of the more prominent workers in the field have been Stahl, of course, and Kaufmann, Demole, Gänshirt, Mangold, Stanley and Seiler. The contributions of these people and many others will be discussed later.

The use of thin-layer chromatography is now almost routine in natural product, pharmaceutical and lipid laboratories, but has not been so well accepted by the organic chemists as it might. The applications to various aspects of nonlipid biochemistry have been hampered by the very polar molecules involved, but these problems are rapidly being overcome by the introduction of new adsorbents.

The field has recently been reviewed again by Demole in French⁷⁹

and English;⁸⁰ by Stahl,³⁷² Schorn³⁴⁶ and Wagner⁴²⁸ in German; by Mangold²³² and Wollish, Schmall and Hawrylyshyn⁴⁵² in English; by Michalec²⁴⁸ and Procházka³¹² in Czech; by Vioque⁴¹⁸ in Spanish; by Kokoti-Kotakis;²⁰⁵ and by Jensen.¹⁷⁶

At present, three books have been published on the subject. Two of these, one edited by Stahl³⁷⁵ with contributions from Bolliger, Brenner, Gänshirt, Mangold, Seiler and Waldi and one written by Randerath,³²⁰ are in German. The third, by Truter⁴⁰⁴ is in English.

GENERAL APPLICATIONS

The motives for the application of thin-layer chromatography are many and varied. Some of these will be considered in the following discussion. The prejudices of an organic chemist with inclinations toward natural product work will be apparent. The various points will all be fairly obvious, but may, on occasion, suggest a solution to a research problem. Examples will be cited to illustrate the discussion, although it should be noted that they are chosen at random and no attempt has been made to cite all of the possible references.

Preliminary Study of a System or Situation

The small amounts of material involved and the speed with which results are obtained make thin-layer chromatography a valuable tool for preliminary explorations of many kinds. The pharmacognosist or plant chemist can learn something about the complexity and, by the judicious choice of spray reagents, even something of the components of a plant extract. He can then use the technique to follow his gross separation procedures and to learn where the products of interest are. The same reasoning applies, of course, to various aspects of plant and animal biochemistry.

The organic chemist, or indeed, any chemist, can explore a large number of reaction conditions, using small amounts of material, in a short time. This applies to both normal synthetic reactions and attempts to degrade a natural product or unknown substance. The operator can readily learn which reactions produce new products and which yield only starting material; which reactions produce complex product mixtures and which do not. If a given material is sought and

is available for comparison, conditions can readily be found which produce it in the best yield and the cleanest form. The author and his co-workers have used this last general idea in the synthesis of the alkaloids, pilocereine³⁸ and anaferine.³³²

Intensive Study of a Single Reaction

After some idea of the best reaction conditions or best degradation scheme has been obtained for a given problem, thin-layer chromatography can be employed to learn considerably more about the reaction. A crude kinetic study of the reaction can be made by removing samples at timed intervals and chromatographing them simultaneously with starting material and the expected product, if available. This may tell whether stable intermediates are involved, whether the desired product is an end product or an intermediate and something of the course of reaction. At least, such a study will tell when all of the starting material is gone and will aid in establishing an optimum reaction time.

The author and his co-workers have used this approach extensively in the structure elucidation of the glucoside, catalposide.⁴⁰ An example from this work is shown in Figure 1.1. Others have used it in the study of the sequential methylation of phenolic acids,²²⁵ the preparation of *p*-bromophenylosazones of xylose derivatives,⁷ the glycerolysis of linseed oil³³⁵ and the biogenetic production of terpenes in mint plants.²⁰ The presence of an unstable intermediate was shown in the synthesis of pseudoionone.²³⁷

The Isolation of Reaction Products

Thin-layer techniques can be applied to the isolation of reaction products in several ways. First of all, if acid-base partition, distillation or crystallization methods are to be used, one can easily learn something of the precision and completeness of the separations and the purity of the various fractions. Furthermore, the short times involved in learning where the product is will satisfy even the most impatient chemist.

If simple separation methods fail and the products must be separated by chromatography, thin layers offer several possibilities. Quantities less than one gram can be separated by preparative thin-



Figure 1.1. Kinetic study of the saponification and epoxide opening of the glucoside catalposide.⁴⁰ The starting material shown on the left at zero time is converted after one hour to a deshydroxybenzoyl derivative (middle spots) which is, in time, converted to a glycol (lower spots). The example clearly shows the presence of an intermediate and indicates the optimum reaction time for the preparation of either product. (Photograph by S. E. Wollman)

layer chromatography. This is, beyond doubt, easier, quicker and more precise than column chromatography and is discussed extensively in Chapter 9. In the author's laboratory, this has proved to be an ideal method for purifying samples prior to a final crystallization for microanalysis. Specific examples of this are noted in the tables in Chapter 11.

Applications to Column Chromatography

The simplest type of column chromatography involves the removal of small amounts of contaminants or tars by passing the sam-

ple, in a suitable solvent, over a short column of adsorbent. Such a process is adaptable to relatively large quantities and gives high yields. Thin-layer techniques can predict, in a short time, the ideal solvent and adsorbent. One needs only to find a system in which the product moves and the contaminant stays at the origin and to transpose this to a short column of the *identical* adsorbent. Normally, the adsorbents used for thin layers are of such small particle size that a pressure system or a vacuum system must be used when they are placed in columns. Such a scheme has been used by Kirchner and Miller¹⁹⁰ for the preparation of terpeneless essential oils. The reverse of this notion, that is, the isolation of small amounts of product from mixtures, has been used to isolate insecticide residues from plant material.

If the quantities involved and the degree of separation required are such that normal column chromatography cannot be avoided, thin-layer techniques can be used to predict the best solvent system and, above all, to analyze the effluent from the column. Several authors have stated or implied^{90,251,365} that the solvents used to give good separations on thin layers can be used directly on a column of the same adsorbent. Duncan⁹⁰ has published the following formula for predicting whether two substances will separate on a column.

$$r = \frac{a}{b + 0.1a}$$

$a = Rf$ of faster moving substance on a thin layer

$b = Rf$ of slower moving substance on a thin layer

Separation can only be expected under the conditions prescribed by Duncan (very high adsorbent-material ratios) when r is greater than unity.

It should be pointed out, however, that all of these situations depend upon the actual magnitude of the thin-layer Rf values. This is particularly true in respect to the Duncan expression which would certainly not be valid when the substances run close to the solvent front. The separations on a column will depend primarily on the retention times of the substances involved and these retention times, as stated for thin-layer chromatography by Zoellner and Wolfram,⁴⁵⁸ are inversely proportional to the Rf values of the substances on the same

adsorbent. Thus, a small difference when R_f values are small can be expected to produce an acceptable difference in retention times and a separation, whereas a large difference when R_f values are close to unity will not give a separation. The author has had some success using the following scheme. A pair of solvents is found which will produce an acceptable separation on a thin layer. The polar component of the solvent system is then reduced until the substances to be separated show R_f 's of about 0.2. This modified system is then used directly on a column of adsorbent identical in every way with that in the thin layer.

The monitoring of the effluent of a column is normally done by analyzing fractions obtained using a fraction cutting device. On a typical large layer (20 by 20 cm), as many as eighteen samples can be analyzed simultaneously. The fractions are then combined according to their components. Such applications are also noted in the tables in Chapter 11, and an example taken from the author's work on catalposide is given in Figure 1.2.

Routine Quantitative Assay

A number of procedures have been published for quantitative assay by thin-layer chromatography. These are discussed in Chapter 10 and documented in Chapter 11. Most of the work has been done in clinical chemistry where speed is an important factor. The major limitation of the technique is the error of about 3 to 5%.

Thin-Layer Chromatography in Conjunction with other Methods

The use of thin-layer chromatography in conjunction with gas chromatography has been especially useful in the lipid field.^{178,232,233,236} Normally, the class separations are made preparatively on thin layers and the classes are resolved, generally after chemical modification, by gas chromatography. In this manner, it is possible to take advantage of the ease of quantification and the high degree of resolution of the latter method. It should be noted, however, that the classes are also often resolved by "reversed phase" thin-layer chromatography.^{181,182,184,230,232 and others}

A method has been suggested¹⁴⁷ for the combined use of thin-layer chromatography and the mass spectrograph. The silica gel taken

2.90 g. of mixture on 180 g. of Silica Gel G

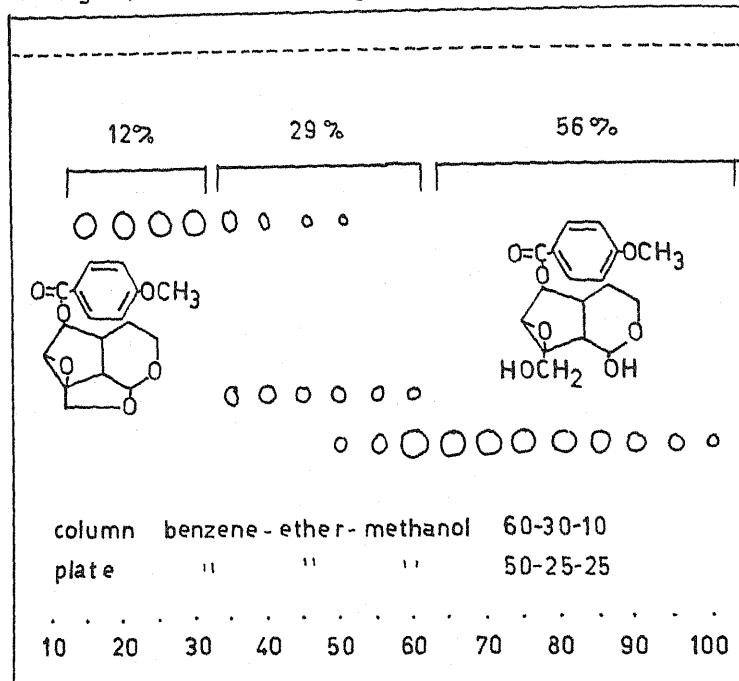


Figure 1.2. A thin-layer analysis of the chromatographic separation of two derivatives of catalposide.⁴⁰ The eluent from a Silica Gel G column was divided using an automatic fraction collector and every fifth tube was examined. The solvents for the column were predicted from thin-layer data as described in the text. The solvent systems used on the column and the analysis layer as well as the structure of the materials isolated are shown. The experiment clearly shows the degree of separation, the relatively short time involved (first sample came off after only fifteen 20 ml fractions) and which fractions contain pure compounds. The center spots represent an, as yet, unknown compound.

from the layer and containing the substance to be investigated is introduced directly into the instrument. The adsorbent does not appear to have any effect on the spectrum. The ratio of sample to adsorbent must be at least 1:100, however, and several micrograms of substance are needed.

The Use of Thin-Layer Patterns

Thin-layer chromatography, like paper chromatography, can be used to determine characteristic component-patterns for drugs, plant

extracts and biochemical preparations. The comparison of these with patterns of adulterated or diseased samples can provide interesting results. Such techniques have been used to study "chemical races" in plants,⁴⁵³ drug contaminants,¹⁶¹ mint oils³²³ and lipid components of multiple sclerosis patients.¹⁵⁷

Classification Systems

In the alkaloids,⁴³⁶ the estrogens²²¹ and mint oil constituents,³²³ thin-layer chromatographic properties have been correlated to some extent with structural types, and classification procedures have been developed. Such systems make it easier to identify known compounds and to obtain information about unknown ones.

CHAPTER 2

Adsorbents

INTRODUCTION

The adsorbents most commonly used in thin-layer chromatography are, in the following order, silicic acid or silica gel,* aluminum oxide or alumina, kieselguhr or diatomaceous earth and powdered cellulose. Those which have been used to a lesser extent are polyamide powders, ion-exchange powders (modified cellulose), "Florisil," calcium sulfate, polyethylene, "Magnesol," hydroxylapatite, "Sephadex," zinc carbonate and various mixtures of these. Kirchner, Miller and Keller¹⁹³ explored several adsorbents using starch and plaster of Paris as binding agents. These data are shown in Table 2.1. Although they pertain only to the separation of essential oils by adsorption processes, they show the large number of substances which can be formed into usable layers. Only calcium hydroxide layers are really poor and, as will be discussed later, the addition of silica gel produces usable layers of this substance. Essentially any substance which has desirable adsorptive and chemical characteristics can be successfully used in thin-layer chromatography.

The four most widely used adsorbents, *silica gel*, *alumina*, *kieselguhr* and *cellulose*, however, offer a wide range of properties and can be used in most cases. Waldi, Schnackerz and Munter⁴³⁶ made a comparative study of Silica Gel G, Aluminum Oxide G and Kieselguhr G with the following conclusions. Silica gel and alumina are, respectively, essentially acidic and basic in character while kieselguhr is

* The major difference between silicic acid and silica gel appears to be solely in the method of preparation. They are chemically identical, but the gel is prepared in such a manner as to enhance its adsorptive power.

TABLE 2.1. CHARACTERISTICS OF CHROMATOSTRIPS MADE
WITH VARIOUS ADSORBENTS^{193*}

| Adsorbent Coating | Physical Characteristics of Strip | Resolution of Essential Oils |
|------------------------|--------------------------------------|------------------------------------|
| Magnesium oxide | soft | none |
| Alumina | excellent | good |
| Alumina + silicic acid | excellent | good |
| Calcium hydroxide | soft, crumbly | none |
| Starch | good | none |
| Dicalcium phosphate | fair | some resolution |
| Bentonite | good | oils decomposed |
| Calcium carbonate | good | slight resolution |
| Magnesium carbonate | fair | slight resolution |
| "Filtrol" | good | separation, but oils decomposed |
| "Filtrol" X202 | good | separation, but oils decomposed |
| "Filtrol," neutral E | good | separation, but oils decomposed |
| "Florisil" | good | fair separation |
| Talc | good | slight separation |
| Silicic acid | excellent | excellent |

* Reproduced from Kirchner, Miller and Keller, *Anal. Chem.*, 23, 420 (1951) through the courtesy of the authors and The American Chemical Society.

neutral. Furthermore, silica gel has the highest capacity (the ability to separate the largest quantity of a mixture) followed by alumina and kieselguhr in that order.

Alumina chromatography is essentially adsorption chromatography; silica gel can function in both adsorption and partition chromatography depending upon the solvent system and kieselguhr is best characterized as a support for partition phenomena. Thus, alumina would be the adsorbent of choice for the separation of non-polar basic or neutral mixtures. Silica gel can be used for nonpolar acidic mixtures and, because of its higher capacity, would even be preferred for the nonpolar neutral materials. Silica gel, kieselguhr and cellulose are all suitable for the separation, through partition processes, of polar molecules. Of course, the properties of these adsorbents can be extensively altered by addition of various acids, bases and buffers so that many purposes can be served which are not covered by the above generalizations.

Although artifact formation on thin layers is rare, it is not unknown. Steroid 16- β -esters have been shown to undergo reactions on alumina layers²⁴⁷ and ethylene ketals have been hydrolyzed on silica gel layers.²⁶³ Alumina in columns is known to catalyze ester hydrolysis, isomerization of double bonds and other reactions.²³² The chromatography of sugars on silica gel layers with ammonia solutions has been shown⁴³⁹ to produce amination reactions.

The various adsorbents can be and are sometimes used in a pure form, but more frequently, they are used in combination with small amounts of added components. These additives can be blended with the solid adsorbent before it is made into layers, or they can be dissolved in the water portion of the slurry which, in most cases, is the vehicle for application to plates. In general the following purposes are served by the additives.

(1) They may serve as binders or substances to make the adsorbent more cohesive and to hold it to the plate. These make the layers much easier to handle and use and make it possible for them to be sprayed with visualizing reagents. The most common binder is plaster of Paris (calcined calcium sulfate, $\text{CaSO}_4 \cdot \frac{1}{2}\text{H}_2\text{O}$) which is added to the dry adsorbent in amounts up to 20 per cent. The first binder used by Meinhard and Hall²⁴⁶ and by Kirchner and his group¹⁹³ was starch, which, although it gives the layers an added stability over plaster of Paris, limits the spray reagents which can be used to visualize the finished chromatograms. Other binders which have been used are polyvinyl alcohol,²⁸⁴ collodion³¹⁷ and an alcohol-soluble polyamide, "Zytel 61."^{*153} Two binders which have been added at later stages are decalin,²⁵⁴ which is added to the developing system and dichlorodimethylsilane,¹⁸⁵ which is applied to the developed plates so that they can be washed like paper chromatograms. The relative advantages and disadvantages of these binders will be discussed in more detail in the section of this chapter devoted to silica gel.

(2) The additives may serve to alter the properties, both physical and chemical, of the adsorbents. Thus, acids, bases and buffers are commonly incorporated into the layers. Reagents which will complex or chelate to a greater or lesser extent with the substances to be sep-

* Du Pont de Nemours, Geneva, Switzerland

arated, are often added. The latter concept is a relatively recent one and will, in the author's opinion, become important.

The impregnation of layers with hydrophobic liquids so that "reversed phase" chromatography can be carried out will be discussed in Chapter 3.

(3) On occasion, materials are added to adsorbents as "fillers." These reduce the activity of the adsorbent and make it somewhat more porous, resulting in shorter developing times. This concept was originally used by Meinhard and Hall²⁴⁶ who added "Celite" (see p. 24) to starch-bound alumina. More recently, Bennett and Heftmann^{26,27} have added Kieselguhr G to Silica Gel G for a similar purpose.

(4) Finally, reagents may be added which will aid in the visualization of the completed chromatogram. The incorporation of phosphors or fluorescent compounds in the adsorbent so that unsaturated compounds can be seen or easily rendered visible in ultraviolet light is a common practice. On occasion, sulfuric acid is added to the adsorbent. This alters the character of the adsorbent and allows the visualization of the completed chromatogram by heating (to char the organic compounds).

Procedures have been developed by Stahl³⁷³ and by Heřmánek, Schwarz and Čekan¹⁴⁴ for relating the activity of alumina layers to the Brockmann⁵⁰ activity scale.

A number of these adsorbents, both modified and pure, are commercially available. These sources will be listed in Table 2.4 at the end of this chapter, although, at the appropriate places, procedures will be given so that an investigator can prepare his own.

Silica Gel or Silicic Acid

Silica gel, used with a plaster of Paris binder is, by far, the most extensively used adsorbent in thin-layer chromatography. It forms a versatile layer which can serve as the solid stationary phase for adsorption chromatography, as a support for the polar liquid phase in normal partition chromatography and as a support for the nonpolar liquid phase in "reversed phase" chromatography. The distinction between the adsorption and normal partition chromatography in this case lies in the solvent system chosen for the chromatogram. If

the system consists of solvents such as hexane, benzene, ether, methanol or similar substances, adsorption phenomena will result. If a system is chosen which contains both a nonpolar liquid and a polar liquid such as water, the result will probably be partition chromatography. In "reversed phase" chromatography, the silica gel layer is impregnated with a nonpolar liquid such as a Silicone oil or a hydrocarbon before use. This will be discussed in Chapter 3.

Two binders, starch and plaster of Paris, are commonly used with silica gel. Both originated with Kirchner, Miller and Keller.¹⁹³ Starch gives a more mechanically stable layer^{8,120,193} which can actually be written on with a blunt lead pencil, but the inclusion of starch in the layer forbids the use of corrosive spray reagents for visualizing the finished chromatogram. (Cold concentrated sulfuric acid may be sprayed on the layers without difficulty, but the sprayed layers cannot be heated to char the spots.) The use of these corrosive spray reagents (sulfuric acid, chromic acid, nitric acid, etc.) as essentially universal reagents for organic substances, is one of the greatest advantages of thin-layer chromatography. On the other hand, the presence of calcium ion (from the plaster of Paris) in the layers has caused some difficulty in the chromatography of inorganic ions³⁵⁵ and nucleotides.³¹⁷

Silica Gel with No Binder. Silica gel has been used only rarely^{1,51,312,322} without some sort of binder. This situation will surely change, however, due to the recent introduction of Woelm "Silica Gel for Thin-layer Chromatography," which contains no binder and depends, for the stability of its layers, on a small particle size. The commercial preparation "Anisil" has no binder as such, but does contain 10 to 15 per cent of magnesium oxide which enhances its layer stability and makes it a basic adsorbent.

A method for the preparation of a silica gel suitable for thin-layer chromatography has been given by Adamec, Matis and Galvánek.¹ The starting material was water glass (mixed, hydrated sodium silicates) and the adsorbent was used without a binder.

Silica Gel with a Plaster of Paris Binder. The commercial preparation Silica Gel G (G for *gips* or gypsum) is of this type. It is manufactured by Merck in Germany according to the specifications of

Stahl and, without doubt, is the most widely used adsorbent in thin-layer chromatography. Mangold²³² has stated that a reasonable approximation of the commercial product can be prepared by thoroughly mixing Mallinckrodt Silicic Acid, 200 mesh, with 10 to 15 per cent of newly calcinated calcium sulfate (prepared by heating reagent grade $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ at 180° for 24 to 48 hours²⁹⁶) of the same grain size. A 200-mesh screen produces particles smaller than 0.12 mm.

Silica Gel G contains appreciable amounts of ferric ion. This does not interfere with the separations of organic compounds, but must be removed for successful work with inorganic ions.³⁵⁵⁻³⁶⁰ Seiler and Rothweiler³⁵⁸ give the following instructions (freely translated) for doing this.

"Five hundred grams of Silica Gel G is treated with 1000 ml of 6*N* hydrochloric acid (500 ml of concentrated hydrochloric acid and 500 ml of distilled water), stirred and allowed to stand. The supernatant liquid, colored yellow by the iron, is decanted and the silica gel is washed twice more with acid and then with three successive 1000-ml portions of distilled water. The adsorbent is then filtered and washed with distilled water until the filtrate is only slightly acidic. It is finally washed with 250 ml of ethanol and 250 ml of benzene and dried in an oven at 120° ."

The material from the above treatment *now contains insufficient binder*. Two grams of plaster of Paris³⁵⁹ or one gram of starch³⁵⁸ are added to 28 g of purified silica gel to obtain a usable adsorbent.

When materials are to be recovered from thin-layer adsorbents by elution, it is suggested^{244,394} that the adsorbent be prewashed. This can be done for Silica Gel G by extracting it three times with boiling methanol²⁴⁴ or by allowing it to stand overnight with the same solvent.³⁹⁴ Honegger found¹⁵⁸ that 50 g of Silica Gel G yielded the following amounts of impurity on elution: with chloroform, 6.8 mg; with benzene, 5.5 mg and with acetone, 10.5 mg. Or, the layers can be prewashed with the developing solvent.

Silica Gel With a Starch Binder. To the author's knowledge, no commercial preparation of this type is available. Kirchner, Miller and Keller¹⁹³ combine 19 g of Merck reagent grade silicic acid, 100

mesh, one gram of "Clinco"^{*} starch, ** 0.15 g of zinc silicate and 0.15 g of zinc cadmium sulfide. The latter two reagents are added as phosphors, but the same proportions would surely serve for the preparation of layers without them. The preparation of starch bound layers is inherently different from the preparation of plaster of Paris bound layers in that the slurry of adsorbent, starch and water is heated before it is applied to plates. This will be discussed in Chapter 3.

Silica Gel with Fluorescing Agents. Several reagents are commonly incorporated into thin layers so that the resulting spots on the developed chromatogram can be seen without using a spray reagent. This is particularly helpful in preparative work. The fluorescing agents, with one exception, are usually blended with the dry adsorbent before the layers are prepared. The exception is sodium fluorescein which is normally dissolved in the liquid used to make the slurry. When the developed plates are placed in short wave ultraviolet light, compounds containing conjugated double bonds show up as dark spots. The various fluorescent materials, the amounts used and their commercial sources are given in Table 2.2.

Acidic Silica Gel. Acids have been incorporated into thin layers for two reasons. The first is to modify the properties of the adsorbent and the second is to aid in visualization. Thus, Stahl³⁶⁹ used 0.5N aqueous oxalic acid rather than pure water to prepare layers. The adsorption activity of such plates is slightly increased.

In contrast to this, Reichelt and Pitra³²² deactivated silica gel by adding 25 per cent water or 43 per cent of dilute (50 per cent) acetic acid to the dry activated powder. In this case, the silica gel was then used without a binder for the separation of cardenolides.

Layers prepared from adsorbents containing small amounts of sulfuric acid can easily be visualized by heating the developed chromatograms on a hot plate or in an oven to char the organic substances. Peifer²⁹⁶ added 2.5 ml of concentrated sulfuric acid to 100 ml of chloroform-methanol, (70:30) v/v, which was subsequently slurried

* Clinton Foods, Inc., Clinton, Iowa.

** Dr. Kirchner has informed the author that still another starch binder is made from ordinary corn starch and "Superior AA Tapioca Flour" mixed in a ratio of 2 to 1. The tapioca flour is a product of Stein, Hall and Co., Inc. of New York. Furthermore, the amount of starch can conveniently be cut in half from the original proportions.

TABLE 2.2. FLUORESCENT AND PHOSPHORESCENT CHEMICALS USED
IN THIN-LAYER CHROMATOGRAPHY

| Name | Amount Used | Commercial Source | Ref. |
|---|----------------------------|---|------|
| 1. Zinc silicate luminescent material P 1, Type 118-2-7 | 2% in adsorbent | General Electric Co., Cleveland, Ohio | 46 |
| 2. Rhodamine 6G | 0.003% in adsorbent | Matheson, Coleman and Bell, East Rutherford, N.J. | 323 |
| 3. Luminescent substance ZS-Super | 2% in adsorbent | Riedel-de Haen, 3016 Seelze bei Hannover, Germany | 117 |
| 4. "Ultraphor" | 0.005% in adsorbent | Badische Anilin and Soda Fabrik, Ludwigshafen (Rhein), Germany | 293 |
| 5. Luminescent chemical No. 601 | 1% in adsorbent | E. I. du Pont de Nemours & Co., Photo Products Dept., Wilmington 98, Delaware | 232 |
| 6. "Sodium fluorescein" | 0.04% sol. in water phase | | 366 |
| 7. Zinc silicate-zinc cadmium sulfide | 0.75% of each in adsorbent | Du Pont phosphors No. 601 and No. 1502 respectively | 193 |

with 50 g of Silica Gel G. The developed chromatograms (in this case on microscope or lantern slides) were visualized by heating.

Basic Silica Gel. Stahl³⁶⁹ described the preparation of basic layers using 0.5*N* aqueous potassium hydroxide rather than water to make the slurry. The layers were found to be somewhat less active than normal. Such plates can be used to separate acids, which stay at the origin, from neutral compounds or bases which move with the solvent. Adsorbents consisting of 80 per cent calcium hydroxide and 20 per cent Silica Gel G⁴⁴⁹ will be more properly considered as a separate adsorbent system. The commercial material "Anisil," as noted above, is a basic adsorbent.

Buffered Silica Gel. In general, buffered silica gel layers have been used for the separation of polar or ionic compounds by partition chromatography. The addition of the buffer is normally made in the same manner as with acids and bases, that is, by using the desired buffer solution in place of water to make the slurry. Such slurries (at least those made with Silica Gel G) must be spread quickly since they thicken more rapidly than usual.²³² The layers should be allowed to stand several hours at room temperature before they are oven-dried.²³²

TABLE 2.3. THE USE OF BUFFERED LAYERS IN THIN-LAYER CHROMATOGRAPHY

| Buffer Sol. | Concentration | Compounds Separated | Ref. |
|---|---------------------------|---|----------------------|
| 1. Sodium acetate | 0.3 m 0.02 m | flavones sugars | 379, 380 307, 377 |
| 2. Monopotassium phosphate and disodium phosphate | equal amounts of 0.2 M | amino acids | 271 |
| 3. Citric acid-phosphate buffers | pH 7 | sugars | 307 |
| 4. Boric acid | 0.1 N | sugars | 307 |
| 5. Ammonium sulfate | 10% | acidic lipids and amphoteric phospholipids | 234 |

This helps to prevent flaking which is also more pronounced with buffered layers. Some of the uses of buffered layers will be summarized in Table 2.3.

The use of boric acid in the separations of sugars is interesting because of the formation of borate complexes between adjacent *cis*-hydroxyl groups. Such complexes are probably responsible for the effectiveness of the buffer.

The use of buffered layers for thin-layer ionophoresis by Honegger¹⁵⁶ is a rather special case since such solutions are normally used to carry current and to form complex ions which can be caused to migrate. Honegger used 0.1 M sodium citrate and acetic acid-formic acid solutions.

Silica Gel Containing Complexing Agents. The addition of complexing agents to thin layers to increase the power of resolution is an intriguing notion and will surely be further developed. At present, the only clear case of this is the use of silver nitrate on silica gel by Barrett, Dallas and Padley¹⁶ and by Morris²⁶⁰ to separate certain closely related lipids. The basis of this separation is the tendency of silver ion to complex with the pi electrons of double bonds to a greater or lesser degree. The former authors used 12.5 per cent silver nitrate to make a slurry with Silica Gel G. A similar result was produced by Morris²⁶⁰ who sprayed layers with a saturated silver nitrate solution or a saturated methanolic boric acid solution (see p. 48) or both.

As previously stated, the use of boric acid layers for sugar separation may well be an example of complex formation. The use of 2,5-hexanedione in the moving phase for the separation of inorganic ions³⁵⁹ is an example of the application of this notion in a reverse manner.

Alumina

Historically, alumina was the first adsorbent used for thin-layer work. Izmailov and Shraiber,¹⁶⁹ Meinhard and Hall²⁴⁶ and the Kirchner group¹⁹³ all used or explored this adsorbent. It is strange, then, that alumina has not been more extensively used than it has. One possible reason for this is that alumina is a more reactive adsorbent than silica gel and is known to catalyze ester hydrolysis, isomerization of double bonds, and other reactions.²³² Also, alumina is a basic substance⁴³⁶ in contrast to the acidic silica gel and has a somewhat lower capacity.⁴³⁶ Thus, one would tend to choose it for the separation of basic or neutral substances only when silica gel separations were poor.

Alumina with No Binder. Again, in contrast with silica gel, alumina has been used to a large extent with no binder. The reasons for this are obscure but probably can be traced to the ready availability of chromatographic grades of alumina and the large body of knowledge which has accumulated on alumina chromatography. Furthermore, layers prepared by simply leveling dry alumina powder on plates give quite good separations. Thus, no slurries or elaborate application apparatus are needed. These layers are mechanically unstable and must be sprayed with caution.⁷⁰

The alumina which is used in this manner is normal, commercially available alumina of any desired activity grade having a particle size of 0.1 mm or 200 to 300 mesh. Recently, Woelm has announced a special "Alumina for Thin-layer Chromatography" which is available in acid, basic and neutral grades and which is more cohesive than usual. This enhanced cohesiveness is produced by smaller particle size.

Huneck¹⁶⁵ has published directions for the preparation of a fibrous alumina which is quite cohesive and which forms stable layers with no binder.

Alumina with a Plaster of Paris Binder. This adsorbent is com-

mercially available as Aluminum Oxide G and as Fluka Aluminum Oxide D5 but, strangely enough, few reports have been made on its application. It has, however, been used to separate alkaloids.⁴³⁶ A reasonable approximation of the commercial product can be prepared²³² by blending Alcoa Activated Alumina,* 200 mesh, with 5 per cent of its weight of plaster of Paris (see p. 19 for preparation of this substance).

Alumina with a Starch Binder. This adsorbent was used by Meinhart and Hall²⁴⁶ in the first recorded use of a bound adsorbent. However, it does not appear to have been used since and would seem to offer no particular advantage.

Kieselguhr or Diatomaceous Earth

Kieselguhr with a Plaster of Paris Binder. Silica gel is acidic and applicable in both adsorption and partition chromatography while alumina is basic and useful in adsorption chromatography. Kieselguhr completes this picture in that it is neutral⁴³⁶ and is primarily a support for the stationary phase in partition chromatography. As such, it has a low capacity and is used for the separation of very polar molecules such as carbohydrates and amino acids. The commercial product, Kieselguhr G, has been used in a buffered form (with sodium acetate) for the separation of the simple sugars³⁷⁷ with reasonable success. Furthermore, it has been impregnated with paraffin^{6,183} and undecane^{181,185,186} for "reversed phase" chromatography.

Kieselguhr Containing Complexing Agents. Halmekoski made slurries of Kieselguhr G with 0.01 mole of sodium molybdate, sodium tungstate or borax (per 30 g of adsorbent and 65 ml of water) and used the resulting layers for the separation of phenolic carboxylic acids.¹³⁶

Cellulose

The use of powdered cellulose in thin layers was essentially an attempt to carry out "paper chromatography" in a short time. This has been successful. The cellulose is, of course, only a support for the stationary liquid phase and has been used in both normal (impregnated with formamide,^{399,400}) and reversed phase chromatography. The capacity of the adsorbent is low.

* Aluminum Company of America, 230 Park Avenue, New York 17, New York

Cellulose without a Binder. The fibrous nature of powdered cellulose permits it to be shaped into stable layers without a binder.^{321,450}

Cellulose with a Plaster of Paris Binder. The commercial product, Cellulose Powder MN 300 G, is of this type. Randerath³¹⁹ has systematically compared this material with paper for the separation of various nucleic acid derivatives. He found that the cellulose layers gave smaller, more compact spots and better separations in a shorter time (90 min) than paper (6 to 8 hr). Wollenweber⁴⁵¹ found similar results with food dyes.

Cellulose Ion-Exchange Powders. Two cellulose powders, modified to produce ion-exchange resins, have been used for the separation of nucleotides.^{316-318,320} These are DEAE-cellulose (diethylaminoethyl cellulose) and ECTEOLA-cellulose (epichlohydrin linking triethanolamine with cellulose). They have been used with a collodion binder^{316,317} or without a binder.³¹⁸

A large number of cellulose powders, plain and modified, bound (G series) and unbound, are commercially available (Table 2.4). In fact, several are available which have not yet been mentioned in the literature.

Ion-Exchange Resins

Berger, Meyniel and Petit²⁹ have reported the use of "Dowex" 1 and "Dowex" 50 (both anion and cation exchangers, 200 to 400 mesh) for the separation of organic and inorganic compounds. They used a 1:1 mixture of the resin and commercial plaster of Paris-bound cellulose, Cellulose Powder MN 300 G.

Polyamide Powders

Polyamide powders are normally used without binders and have been developed by two groups. Davídek and his co-workers^{69,71,74} have spread commercially available polyamide powder on plates and used the resulting layers for the separation of such polar molecules as flavonoids and gallic acid esters. Wang and his co-workers^{219a,436a} allowed 20 per cent solutions of ϵ -polycaprolactam resin in 80 per cent formic acid to evaporate on glass plates and used the resulting thin layers for the separation of phenols.

A commercial product is now available from Woelm (see Table 2.4).

Hydroxyl-Apatite

Hofmann has reported the use of hydroxyl-apatite (a complex calcium phosphate hydroxide*) for the separation of 1- and 2-mono-glycerides¹⁵⁵ and of proteins.¹⁵⁸ In the former case, he used plaster of Paris as a binder and in the latter case, "Zytel 61" (see p. 16).

"Celite"

"Celite" No. 545 with a plaster of Paris binder has been used¹¹⁴ for the separation, by partition processes, of steroids. The layers were impregnated with formamide. Such layers have extremely short developing times (3 to 7 minutes).

"Sephadex"

"Sephadex" G 25, a cross-linked dextran which differentiates according to the molecular weight of the molecules being separated, has been used for the chromatography of proteins.⁸² A modified adsorbent, "DEAE-Sephadex" has been used for the separation, by gradient elution, of proteins and nucleotides.⁸²

Polyacrylonitrile "Perlon" Mixtures

Mixtures of polyacrylonitrile and "Perlon" (a polycaprolactam powder) have been used, in a buffered state, to separate anthocyanins and such very polar molecules as sugars.³⁶

Zinc Carbonate

Zinc carbonate with 5 per cent starch as binder has been used for the separation of 2,4-dinitrophenylhydrazones.⁹

Polyethylene Powder

Polyethylene powder, "Hostalen S," has been used²³² for the separation of fatty acids and their methyl esters.

Calcium Sulfate as Adsorbent

Layers prepared from pure calcium sulfate have been used for the separation of steroids²⁴³ and lipids.¹⁸⁰ The major advantage of such

* Prepared according to Anacker and Stoy^{4b}

plates is that they can be mechanically washed like paper chromatograms. Thus, even more spray reagent systems can be used.

"Magnesol" (Adsorptive Magnesium Silicate)

This adsorbent with a starch binder was preferred by Bryant⁵¹ and by Pryor and Bryant³¹³ for the separation of the essential oils. A commercial product containing no binder but especially prepared for thin-layer work is available.

Calcium Hydroxide with Silica Gel G

Layers prepared from a mixture of calcium hydroxide and Silica Gel G (6:1) and (4:1) have been found to produce good separations in the carotenoid field.^{166,449}

"Florisil" (Mixed Silica Gel and Magnesia)

Peifer²⁹⁶ has described a procedure for the preparation of micro-chromatoplates from "Florisil"-plaster of Paris (see p. 46).

CONCLUSION

Obviously, any finely divided material having the desired adsorptive, structural or chemical properties can be used as an adsorbent for thin-layer chromatography. Probably, the area which will be developed most fruitfully is the use of mixed adsorbents. Several of these are given above, and others, such as Silica Gel G-Kieselguhr G mixtures, have been reported.^{26,27}

It is rather interesting that the one factor which delayed the development of this technique, that is, commercial availability of materials, has now progressed so far that several adsorbents are available (the various types of cellulose) for which no published uses are recorded.

The commercial sources of most of the adsorbents are given in Table 2.4 and the specific adsorbents which have been used for specific separations are detailed in Chapter 11.

Thin-Layer Chromatography

TABLE 2.4. COMMERCIAL SOURCES OF THIN-LAYER CHROMATOGRAPHY ADSORBENTS

| Commercial Name | Nature | Manufacturer | U. S. Source |
|--|--|--|--|
| "Anisil", Silica Gel Woelm | silica gel-magnesium oxide pure silica gel | Analytical Co., Hamden, Conn. M. Woch, Eschwege, Germany | Alupharan Chemicals, 616 Commercial Pl., P.O. Box 755, New Orleans, La. |
| Silica Gel G | silica gel with plaster of Paris | E. Merck, A. G., Darmstadt, Germany | Brinkmann Instruments, 115 Cuttermill Rd., Great Neck, N.Y. |
| Silica Gel H Silica Gel HF | pure silica gel pure silica gel with inorganic fluorescent material | E. Merck E. Merck | Brinkmann Instr. Brinkmann Instr. |
| Silica Gel GF | silica gel with plaster of Paris and in- organic fluorescent material | E. Merck | Brinkmann Instr. |
| Silica Gel D5 | silica gel with 5% plaster of Paris | Fluka, A. G., Buchs, Switzerland | Gallard - Schlesinger, Chemical Mfg. Corp., 1001 Franklin Ave., Garden City, N.Y. |
| Aluminum Oxide DO Alumina Woelm Basic Alumina Woelm Neutral Alumina Woelm Acidic Aluminum Oxide G Aluminum Oxide D5 Aluminum Oxide D5F | pure aluminum oxide pure basic aluminum oxide ¹ pure neutral aluminum oxide ¹ pure acidic aluminum oxide ¹ aluminum oxide with plaster of Paris aluminum oxide with 5% plaster of Paris aluminum oxide with 5% plaster of Paris and a fluorescence indicator | Fluka Woelm Woelm Woelm E. Merck Fluka Fluka | Gallard-Schlesinger Alupharan Alupharan Alupharan Brinkmann Instr. Gallard-Schlesinger Gallard-Schlesinger |
| Kieselgeln G Cellulose Powder MN 300 | diatomaceous earth with plaster of Paris pure cellulose powder | E. Merck Macherey Nagel & Co. | Brinkmann Instr. Brinkmann Instr. |
| Cellulose Powder MN 300G | cellulose powder with plaster of Paris | Macherey, Nagel & Co. | Brinkmann Instr. |
| Cellulose Powder MN 300 F254 | cellulose powder with an inorganic fluores- cent material | Macherey, Nagel & Co. | Brinkmann Instr. |
| Cellulose Powder MN 300/AC | cellulose powder with plaster of Paris and an inorganic fluorescent material pure acetylated (10%) cellulose | Macherey, Nagel & Co. | Brinkmann Instr. |

| | | | |
|--|--|---|------------------|
| Cellulose Powder MN 300 G/AC | acetylated (10%) cellulose with plaster of Paris | Macherey, Nagel & Co. | Brinkmann Instr. |
| Cellulose Powder MN 300 CM | pure carboxymethyl cellulose Paris | Macherey, Nagel & Co. | Brinkmann Instr. |
| Cellulose Powder MN 300 G/CM | carboxymethyl cellulose with plaster of Paris | Macherey, Nagel & Co. | Brinkmann Instr. |
| Cellulose Powder MN 300 P Cellulose Powder MN 300 | pure cellulose phosphate with plaster of Paris | Macherey, Nagel & Co. | Brinkmann Instr. |
| G/P | cellulose phosphate with plaster of Paris | Macherey, Nagel & Co. | Brinkmann Instr. |
| Cellulose Powder MN 300 DEAE | pure diethylaminoethyl cellulose | Macherey, Nagel & Co. | Brinkmann Instr. |
| Cellulose Powder MN 300 G/DEAE | diethylaminoethyl cellulose with plaster of Paris | Macherey, Nagel & Co. | Brinkmann Instr. |
| Cellulose Powder MN 300 ECTEOLA | pure anion exchange cellulose | Macherey, Nagel & Co. | Brinkmann Instr. |
| Cellulose Powder C-1000 | cellulose powder with plaster of Paris | Excorna O. H. G., Mainz, Germany | Brinkmann Instr. |
| Magnesium Silicate Woelm | pure magnesium silicate ¹ | Woelm | Alupharin |
| Polyamide MNP | pure polyamide powder | Macherey, Nagel & Co. | Brinkmann Instr. |
| Polyamide Woelm | pure polyamide powder ¹ | Woelm | Alupharin |
| “Hostalen S” | Polyethylene powder | Farwerke Hoechst, A. G., Frankfurt a.M., Germany | |
| “Florisil” | activated magnesium silicate | Floridin Co., P.O. Box 989, Tallahassee, Fla. | |
| “Sephadex” | cross-linked dextrans | Deutsch. Pharmacia, G.m.b.H., Frankfurt a.M., Germany | |
| Polyacrylonitrile | | Farbenfabriken Bayer, Dormagen, Germany | |
| “Perlon” | | Farwerken Hoechst, Bo- bingen, Germany | |
| ε-Polycaprolactam resin | | Toyo Rayon Co., Nakano- shima, Kita-ku, Osaka, Japan | |
| “Dowex” 1 and 50 | | Dow Chemical Co., Mid- land, Mich. | |
| “Celite” No. 545 | anion and cation exchangers diatomaceous earth | Johns-Manville, Interna- tional Corp., New York | |

¹ These adsorbents are of an especially fine particle size for thin-layer chromatography and produce mechanically stable layers without binders.

CHAPTER 3

Preparation of Thin Layers

INTRODUCTION

The thin layers of adsorbent used for thin-layer chromatography are supported and held in place by glass plates. The plates may, in theory, be any convenient size, but in fact the size is usually dictated by the apparatus used to prepare the layers. Layers are applied to the plates by spreading or spraying a slurry, by spreading a dry powder, or in the case of small plates, by dipping them in a slurry. The thin film of slurry is dried to produce the thin layer. The layer may then be treated in various ways before it is used.

THE VARIABLES IN LAYER PREPARATION

In general, the variables to be considered are the nature of the adsorbent (Chapter 2), the type of glass support plate, the thickness of the layer and the moisture content.

The Glass Plates

The glass plates used in thin-layer chromatography may be flat and smooth, flat and lightly ground^{180,254,328,334} or ridged as shown in the cross-section diagram in Figure 3.1. Flat, smooth plates have been almost universally used, but the ground surfaces are said to increase the adhesion of the layers. The ridged plates are used solely to simplify layer preparation. Thus, one simply pours the adsorbent-water slurry on a ridged plate, wipes off the excess with a spatula and dries the finished layer. The squared-ridge plates (Figure 3.1a) have been advocated by Gamp, Studer, Linde and Meyer,¹¹⁴ but the rounded ones (Figure 3.1b) are more readily available from normal glass shops,

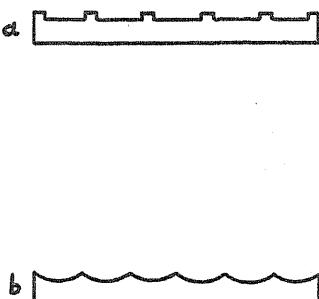


Figure 3.1. Cross-section views of two types of ridged glass plates.

where they are used to make semiopaque windows.* The ridged plates and the microchromatoplate system described later (p. 42) probably offer the most convenient systems for student use. To prevent accidents, all plates should have slightly beveled and smoothed edges.

The size and thickness of the glass plates are essentially dictated if a mechanical device such as those described below is chosen to prepare the layers. It has been found convenient to use, routinely, at least two sizes (exclusive of microplates). The smaller size should be wide enough to carry out concurrent, one-dimensional chromatography on three to four samples (about 2 in. or 5 cm minimum). Most workers allow the solvent front to rise 10 to 15 cm so that an over-all length of 20 cm or 8 in. is satisfactory. The larger size should be a square plate suitable for two-dimensional chromatography of a single sample or simultaneous, one-dimensional chromatography of eighteen or more samples. In a commercial apparatus system, the dimension of the square plate is equal to the length of the small plate (20 cm or 8 in.) and a multiple of its width. Such two-size systems are available with Stahl-type apparatus from Desaga-Brinkmann (p. 37) (5×20 cm and 20×20 cm) and Research Specialties Co. (p. 40) (2×8 in. and 8×8 in.). The Kirchner-type** apparatus manufactured by

* The author's attention was first called to the use of rounded plates by Dr. Louis Long of the Quartermaster Research and Development Laboratories in Natick, Mass.

** The author has elected to give this name to the type of apparatus in which the glass plate is passed under a stationary hopper because the Kirchner group was the first one to describe such an apparatus.^{25a}

Camag (p. 36) is normally designed for only one width, but two sizes of apparatus (10 cm and 20 cm width) are available. On the other hand, the Kirchner-type apparatus can be used with any type and thickness of glass, while the Stahl design works best with the plates supplied by the manufacturer. Although soft glass plates are routinely used, "Pyrex" plates are available from the manufacturers of the spreading apparatus.

If layers are prepared by spraying or by any of the simpler techniques discussed below, the plates can be of any size or type.

The original "chromatostrips" of Kirchner, Miller and Keller¹⁹³ were only wide enough for one sample (0.5 in.) although these workers also used wide plates. Such small strips are prepared in an apparatus designed by Miller and Kirchner²⁵³ and are still used³⁸⁸⁻³⁸⁹ by Stanley and his co-workers. However, they preclude a concentration study in which the sample is applied to a single plate in several concentrations, as well as a more precise comparison of two or three samples. The narrow plates are, however, more suitable for descending chromatography (p. 68).

The use of microscope slides for the preparation of "microchromatoplates" was described by Meinhard and Hall²⁴⁶ in the first paper advocating the use of bound layers. Lately, the use of such plates has again become fashionable^{152,487} and Peifer,²⁹⁶ in an exceptionally fine paper, has described a complete system based upon microscope and lantern slides (see p. 42).

A somewhat different type of layer support was worked out by Lie and Nyc,²¹⁹ who coated the inside of test tubes with thin layers of adsorbent. Rosi and Hamilton³³⁰ have prepared layers on both sides of glass plates so that they may be utilized more efficiently.

The Thickness of the Layer

Within a reasonably wide range (0.15 to 2.0 mm) the thickness of the layers is unimportant with respect to *Rf*'s and the degree of separation. The thickness itself should be prescribed by the type of information or results sought. For diagnostic or qualitative work, very thin layers are best because the spray reagent is much more sensitive when it does not have to "search" for tiny amounts of substance in a large amount of adsorbent. On the contrary, when the work is pre-

parative in nature, it is more efficient to use as thick a layer as possible so that the maximum amount of material can be separated in a single chromatogram.

The minimum thickness of about 0.15 mm was determined by Stahl, Schröter, Kraft and Renz³⁸¹ through a study of *Rf* vs. thickness. Only above this value were the *Rf*'s relatively constant (on silica gel layers). The maximum thickness is determined by the difficulty of drying thick layers without cracking and the degree to which there is unequal migration on the top and bottom of a layer. Honegger¹⁵⁸ investigated layers up to 5 mm thick and concluded that the best results were obtained between 1 and 3 mm. Ritter and Meyer,³²⁹ in a similar study, obtained better results with 1-mm layers than with 2-mm layers. The vast majority of diagnostic work has been done with layers 0.25 mm thick because the original Stahl-Desaga apparatus produced layers of this thickness. The Kirchner group¹⁹³ used 0.02 in. (0.51 mm).

Thin layers of unbound adsorbents which are prepared by spreading the dry powders on a plate are generally somewhat thicker, ranging from 0.5 mm²⁸⁰ to 1 mm.⁵²

The Moisture Content

The activity of adsorbents to be used for adsorption chromatography is greatly reduced by small amounts of water. Since most thin layers are prepared from aqueous slurries, this variable is controlled largely by the length of time and the temperature used to dry and activate the layers. This will be discussed in detail in a later section entitled "Drying of Layers" (see p. 46).

LAYER PREPARATION

Cleaning the Glass Plates

It is essential that the glass plates be as clean as possible and that they be particularly free of grease. Normally, washing the plates with detergent and wiping them with cotton soaked with hexane just before application of adsorbent will suffice. Sometimes, however, it is necessary to clean the plates with some type of cleaning solution. Peifer²⁹⁰ has recommended that his microchromatoplates be washed

successively with detergent solution, water and finally with 50 per cent methanol-water slightly acidified with hydrochloric acid.

The Preparation of Slurries

As previously stated, the majority of thin-layers are prepared by spreading a uniform film of a slurry of the adsorbent on glass plates and allowing it to dry. Normally, the liquid portion of such a slurry is water, which may contain acids, bases, buffers or complexing agents, and the solid portion is adsorbent and binder. The major problem associated with these slurries is the obtaining of a viscosity suitable for the manner of spreading. If the slurry is too thin, it runs through the spreader too rapidly and produces excessively thin layers. When it is too thick, it does not run through the spreader rapidly enough and may clump.

When plaster of Paris is used as a binder, this viscosity is controlled by two factors: the relative amounts of adsorbent and water, and the time elapsing between the addition of water to adsorbent and the actual spreading operation. The hydration of plaster of Paris ($\text{CaSO}_4 \cdot \frac{1}{2}\text{H}_2\text{O}$) to gypsum ($\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$) is so rapid that the slurry becomes unmanageable in two to three minutes. When buffers or other modifying reagents are added to the slurry, this time is appreciably shortened.²³² The actual mixing of adsorbent and water can be carried out by shaking them in a stoppered flask, by triturating them in a mortar or by stirring them in some other vessel. Table 3.1 shows the amounts of water suggested for various commercially available adsorbents. When possible, the type of spreading device is also given. Commercial sources of the adsorbents have been cited in the preceding chapter.

For the preparation of very thick layers, Honegger¹⁵⁸ suggests that these ratios be slightly modified by decreasing the amount of water. Furthermore, for layers from 3 to 5 mm thick, he added an additional 2 per cent of plaster of Paris and dried the layers under an infrared lamp. His data are given in Table 3.2.

The procedure for preparing a slurry containing starch as a binder is essentially different in that it involves a heating step. Kirchner, Miller and Keller¹⁰³ give the following procedure for such a preparation containing phosphors.

"The specified amounts of material [19 g of Merck reagent grade

TABLE 3.1. RECOMMENDED AMOUNTS OF WATER TO BE ADDED TO VARIOUS COMMERCIAL ADSORBENTS

| Adsorbent | Adsorbent-water, w/v, g/ml | Apparatus | Ref. |
|---------------------------------------|---|-----------|--------|
| Silica Gel G | 30/60 | Desaga | Manuf. |
| Silica Gel Woelm ¹ | 30/45 | — | Manuf. |
| Silica Gel D5 | 20/50 | Camag | Manuf. |
| Silica Gel, "Anisil" | 15/30 | Desaga | Manuf. |
| Aluminum Oxide G | 25/50 | Desaga | 232 |
| Aluminum Oxide D5 | 20/65 | Desaga | 232 |
| | 20/50 | Camag | Manuf. |
| Aluminum Oxide D5F | 20/50 | Camag | Manuf. |
| Alumina Woelm ^{1, 2} | 35/40 | — | Manuf. |
| Kieselguhr G | 25/50 | Desaga | 377 |
| Cellulose MN 300 G | 15/100 | Desaga | 319 |
| Magnesium Silicate Woelm ¹ | 15/45 | — | Manuf. |
| Polyamide Woelm ¹ | 5 g in 45 ml of chloroform-methanol (2:3) | — | Manuf. |

¹ These adsorbents contain no binder, but are of an especially small particle size.² Same proportions for acid, basic or neutral.TABLE 3.2. HONEGGER'S DATA FOR PREPARATION OF VERY THICK LAYERS¹ *

| Adsorbent | Thickness, mm | Adsorbent-H ₂ O, g/ml | Air-dry, min | Infrared dry- ing, min |
|------------------|------------------|-------------------------------------|--------------|---------------------------|
| Silica Gel G | 1-1.5 | 1:1.7 | 5 | — |
| | 2-3 | 1:1.6 | 30 | 30 |
| | 3-4 ² | 1:1.6 | 30 | 30 |
| | 4-5 ² | 1:1.57 | 30 | 30 |
| Aluminum Oxide G | 1-1.5 | 1:1 | 5 | 30 |
| | 4-5 | 1:0.9 | 10 | 30 |

¹ The data were obtained using the Camag apparatus.² Added 2% more plaster of Paris.* Reproduced from Honegger, *Helv. Chim. Acta*, 45, 1409 (1962) through the courtesy of the author and Verlag Helvetica Chimica Acta, Basel, Switzerland.

silicic acid passing 100 mesh sieve, 1 g of "Clinco 15" starch, 0.15 g of zinc silicate and 0.15 g of zinc cadmium sulfide] thoroughly blended while dry, were mixed with 36 ml of distilled water in a 250 ml beaker. The slurry was then heated on a water bath held at 85° with constant stirring until it thickened (1.75 minutes) and was then held at this temperature for 30 seconds longer with stirring. The beaker was removed from the bath and 2 to 7 ml of water were added immediately to form a thin paste." As stated on p. 20, a mixture of corn

starch and "Superior AA Tapioca Flour" is better than "Clinco" starch. If the amount of starch is decreased by half, it is not necessary to watch the temperature during the heating. The mixture must only be warmed thoroughly to thicken it.

Although water is the most common liquid for the preparation of slurries, several workers have preferred nonaqueous systems. In addition to the test-tube system of Lie and Nyc²¹⁹ and the micro-chromatoplate system of Peifer,²⁹⁶ which are quoted elsewhere in this chapter, Hörhammer, Wagner and Bittner¹⁶⁰ have used ethyl acetate, Duncan⁹⁰ has used water-methanol, 1:1, v/v, and Müller and Honerlagen²⁷⁰ have used acetone. None of these authors used the conventional spreading apparatus.

Mechanical Devices for the Preparation of Layers

At the present time, these devices fall into three major categories, the Kirchner type (see footnote on p. 31), the Stahl type and the spray type. While these "gadgets" are considerably more expensive and complicated than those used in some of the methods which will be described later, the author believes they have a distinct advantage. They produce uniform layers of any desired thickness in a very efficient manner.

The Kirchner Type. This type of spreading device is characterized by a stationary reservoir or hopper which deposits a thin film of adsorbent slurry on glass plates which are slowly passed under it. The layer-thickness is determined by the distance between the plate and the back edge of the hopper, and the width of the plates is predetermined by the size of the apparatus. The first such apparatus was designed by Miller and Kirchner in 1954²⁵³ to produce "chromatostrips" 0.5 in. wide. The apparatus has since been modified by Applewhite, Diamond and Goldblatt⁸ and shop drawings of the modification are available from the authors (Western Regional Research Laboratory, U. S. Department of Agriculture, Albany, Calif.).

A commercial model of this type of apparatus is shown in Figure 3.2 and is manufactured by Camag* in Switzerland. It is based upon the design of K. Mutter and J. F. Hofstetter at Hofmann-LaRoche A.

* Camag A. G., Homburger Str. 24, Muttenz, B. L., Switzerland. U. S. representatives: A. H. Thomas Company, Vine Street at 3rd, Philadelphia, Pa. and Microchemical Specialties Co., 1825 East Shore Highway, Berkeley 10, Cal.

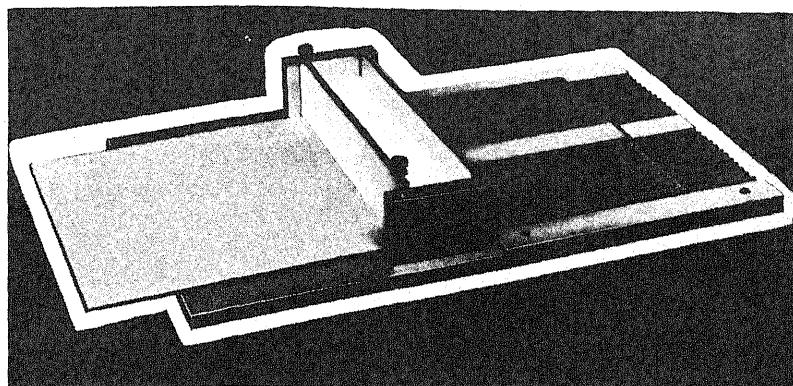


Figure 3.2. The commercial model of the Kirchner-type apparatus as designed by Mutter and Hofstetter. (Reproduced through the courtesy of Camag A. G.)

G. in Basel. Two models of this apparatus are available; one of these (Model A) coats plates 20 by 10 cm for one-dimensional chromatography and the second (Model B) coats plates either 10 cm or 20 cm in width. The Camag apparatus has been slightly modified by Wollish, Schmall and Hawrylshyn.⁴⁵² A similar apparatus is manufactured by the Kensington Scientific Corp.* in California.

It would appear to the author (who has had no experience with this apparatus) that the Kirchner type would be somewhat more tedious to use and less flexible with respect to plate width, than the Stahl type. On the other hand, it is less sensitive to plate thickness and probably yields more consistently uniform layers on small plates.

The Stahl Type. This type of spreading device is characterized by a moving slurry reservoir and stationary plates. The plates are arranged in a continuous surface on a support (the commercial models use plastic slabs) having a raised edge on two sides to hold the plates in position during the coating operation. The reservoir, machined in such a way that it produces the desired layer thickness, is then passed over the plates.

The first commercial model of this design was worked out by Stahl and is manufactured by Desaga** in Germany. The apparatus is pic-

* Kensington Scientific Corp., 1717 Fifth St., Berkeley, Cal.

** C. Desaga, G.m.b.H., Hauptstrasse 60, Heidelberg, Germany. U. S. representative: C. A. Brinkmann and Co., Inc., 115 Cutter Mill Rd., Great Neck, N.Y.

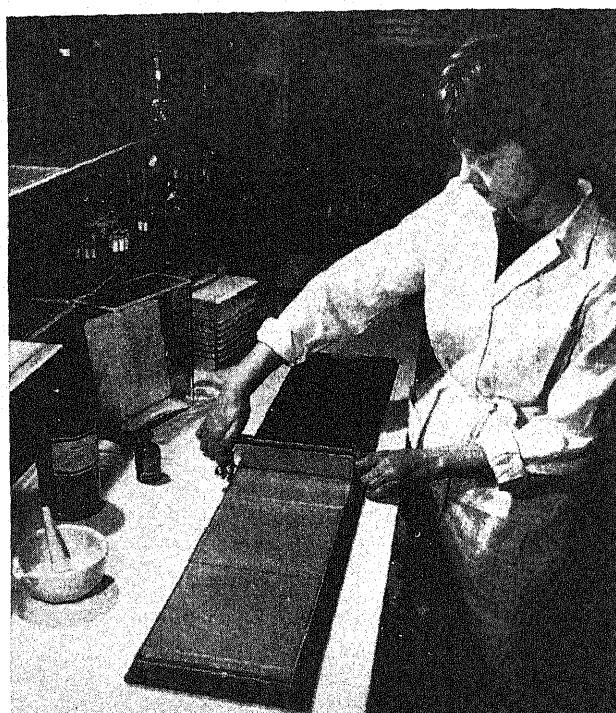


Figure 3.3. The Stahl-Desaga apparatus showing, in detail, the variable thickness applicator, a, and the standard applicator, b. (Reproduced through the courtesy of Brinkmann Instruments, Inc.)

tured in Figure 3.3. The spreader is available in two models; the Standard Model which gives layers 0.25 mm thick and the Model S 11 which gives layers of any thickness up to 2 mm. The standard plate sizes are 5×20 cm and 20×20 cm. Twenty of the small plates or five of the large ones can be coated in a single operation. While the technique works beautifully with the larger plates, the author has had difficulty obtaining uniform layers on small plates. This difficulty has also been encountered by Bennett and Heftmann²⁵ who solved it by placing a series of the large size plates (20×20 cm) under the small ones.* A glass slab or a smooth desk top can also alleviate

* Shandon Scientific Co., Ltd., 65 Pound Lane, London, N.W. 10 (U.S. representative, Consolidated Laboratories, Inc., P.O. Box 234, Chicago Heights, Ill.) manufactures an apparatus to avoid this difficulty. It contains a special leveling device and layer thicknesses are guaranteed to ± 0.010 mm.

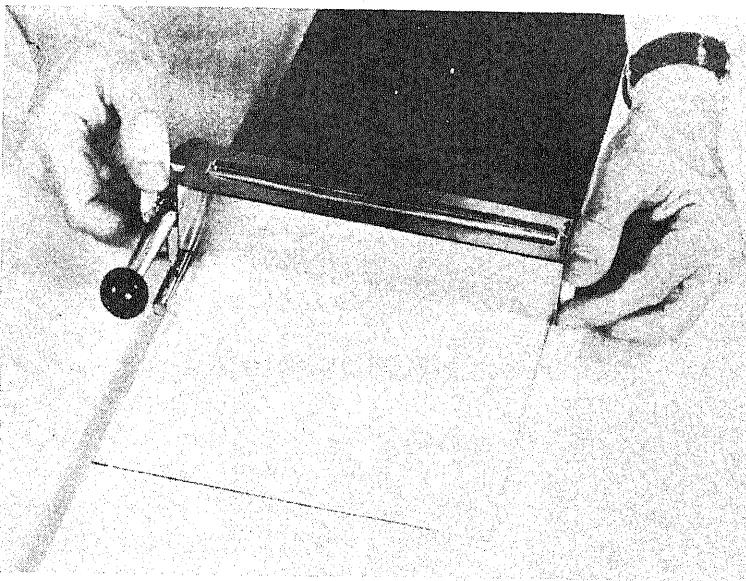


Figure 3.3a.

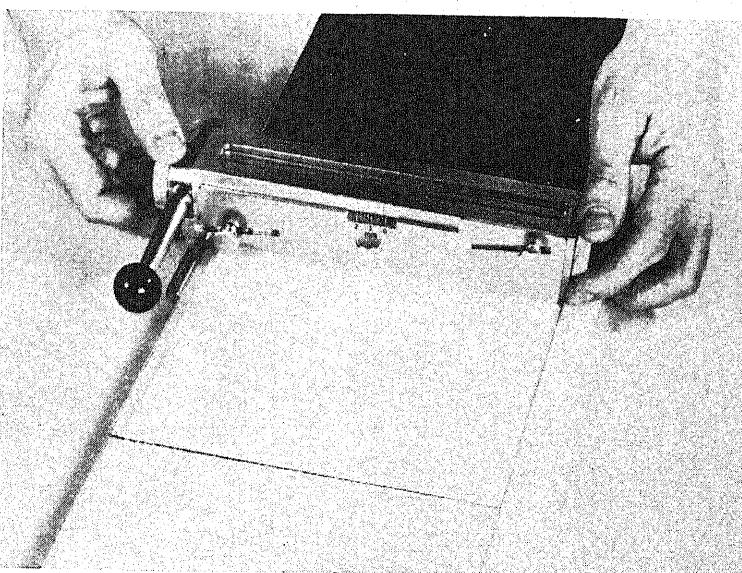


Figure 3.3b.

the problem. Sometimes wavy layers are produced by the "bump" caused by going from one plate to the next. As long as the developing solvent is passed in a direction parallel with the spreading operation, this is no problem.

A second commercial model similar to the Desaga apparatus is manufactured in the United States by Research Specialities Co.* This apparatus can also be purchased in a standard model or a variable thickness model. It is shown in Figure 3.4.

Home-made apparatus of the Stahl type has been devised by Barbier, Jäger, Tobias and Wyss,¹³ Machata,^{227**} Gamp, Studer, Linde and Meyer¹¹⁴ and Schulze and Wenzel.³⁴⁹

Sprayed Layers. The notion of spraying a thin slurry of adsorbent on glass plates has been suggested, notably by Reitsema,³²³ who used a small paint sprayer. Bekersky²⁴ has worked out a procedure using a normal laboratory spray bottle. Dilute slurries of Silica Gel G (15 g to 35 ml of water) or Aluminum Oxide G (20 g to 50 ml of water) are sprayed from a distance of 7 to 9 in. onto plates in a horizontal position. The method produces fairly uniform smooth layers, but the final thickness is unknown and only approximately controllable. The method has not yet been commercialized.

"Poor Man's Layers"

The development of a cheap method for the preparation of uniform, thin layers has challenged the ingenuity of chemists everywhere. This has resulted in the evolution of several acceptable methods. While the author is a firm believer in the more complex spreaders, these methods certainly offer possibilities for student use and for persons who wish to explore the system before investing in an apparatus.

The simplest way of preparing a layer of slurry is surely that of Lees and DeMuria²¹⁴ as shown in Figure 3.5. One to three layers of tape, either surgical or masking, but not "Scotch," are put on two opposite edges of a single glass plate or on the outside edges of the outside plates of a set arranged on a flat surface. The slurry is poured

* Research Specialty Co., 200 South Gerrard Blvd., Richmond, Calif.

** A plastic apparatus of this type is manufactured by Applied Science Laboratories, College Park, Penn.

on an open end and smoothed by drawing a glass rod over the plate or plates in a direction parallel with the taped edges. The tape supports the rod and determines the layer thickness. After a few minutes, the tape is removed and the layers are dried. The method is not very satisfactory for preparing thick (ca. 1–2 mm) layers. A similar method was devised by Duncan⁹⁰ who used removable metal strips on the edge of the plates to support the leveling rod. In this case, the thickness of the metal determines the thickness of the layer. Duncan used methanol-water, 1:1, v/v, to prepare his slurries. The detail of such a strip is shown in Figure 3.6. Boll⁴¹ arranged a set of plates in an apparatus with raised edges. The slurry is poured on and leveled with a straight edge. Still another method is to machine out an indentation in a large spatula to a depth corresponding to the desired thickness. Such a spatula (Figure 3.7) is then used to level a slurry on a glass plate which is large enough to support the two ends of the blade.* As in many other aspects of thin-layer chromatography, the first mention of this type of thing was made by Kirchner, Miller and Keller¹⁸³ who used an arrangement of glass plates, some placed 0.02 in. above the others and serving as support for a leveling rod. Thus, this concept is as old as the method.

Still other workers prefer to pour a slurry of adsorbent in ethyl acetate on a plate and to level it by tipping it.¹⁶⁰ Such layers are said to require no activation and to function satisfactorily in a chamber which is not saturated. The same procedure has been used with aqueous starch-silica gel slurries.⁴³ A similar scheme was used by Wang to prepare polyamide layers.^{436a} He prepared 20 per cent solutions of polyamide in 80 per cent formic acid, poured these solutions on glass plates, leveled them by tipping and allowed them to dry.

A special problem arises with the preparation of thin layers on the inside walls of test tubes.²¹⁹ Such layers were prepared and used by the following method. The test tube to be coated (13.5 × 150 mm) is filled with a suspension of silicic acid (no binder) and chloroform (1:2.5, w/v) and allowed to stand for about 5 seconds. The contents are then slowly poured out of the tube in one continuous slow tilting motion. After most of the slurry is discharged, the vessel is held in an

* This method was mentioned to the author by Mr. Robert Conkin of the Monsanto Chemical Co., St. Louis, Mo.

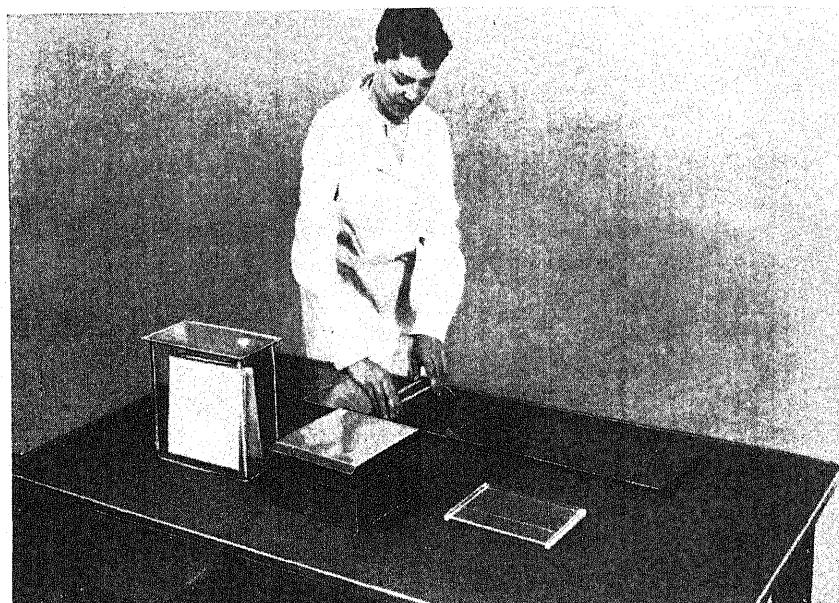


Figure 3.4. The Research Specialities apparatus showing, in detail, the variable thickness applicator, a, and the standard applicator, b. (Reproduced through the courtesy of Research Specialities Co.)

inverted position for 2 to 3 seconds and then slowly tilted, without rotation, to a horizontal position for drying. The top portion (in the horizontal position) is marked because it is the linear surface where the sample will be placed. An additional coating is placed on the open end of the test tube by immersing the tip end about 5 mm, at an angle of 45°, in the adsorbent slurry. In this position it is rotated once. The sample is placed 1 cm from the open end on the side previously marked. The open end is dipped in a suitable developing solution.

Microchromatoplates

Thin layers have been prepared on microscope slides and lantern slides by spreading a paste with a spatula²⁴⁶ with an especially designed gadget (for microscope slides only)⁴³⁷ and using the Stahl apparatus with an alternate arrangement to hold the slides during the slurry application.¹⁵² The author has prepared satisfactory layers using the complete Stahl assembly.

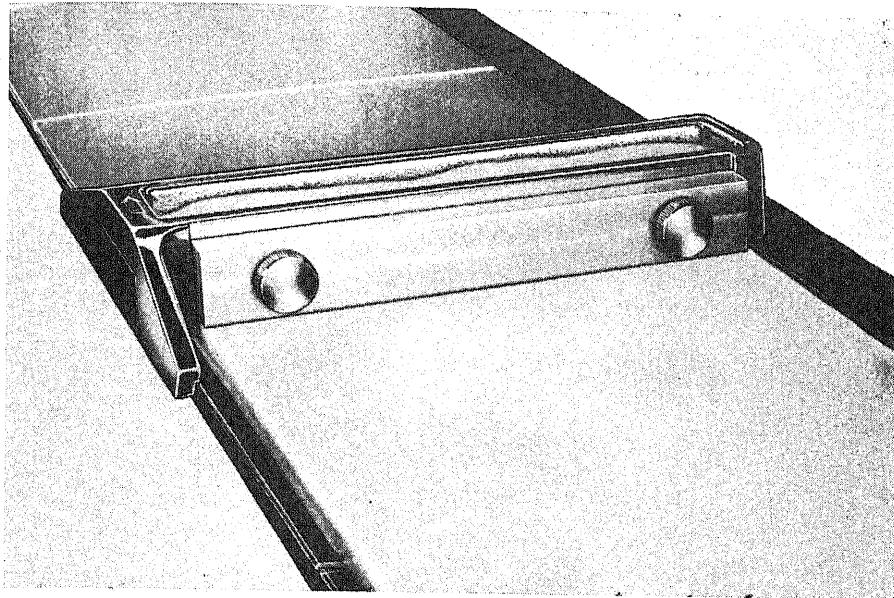


Figure 3.4a.

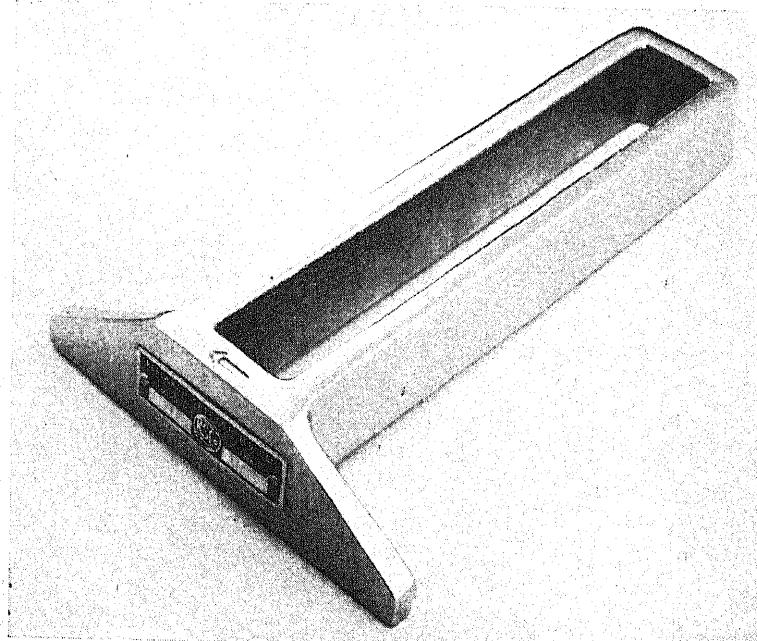


Figure 3.4b.

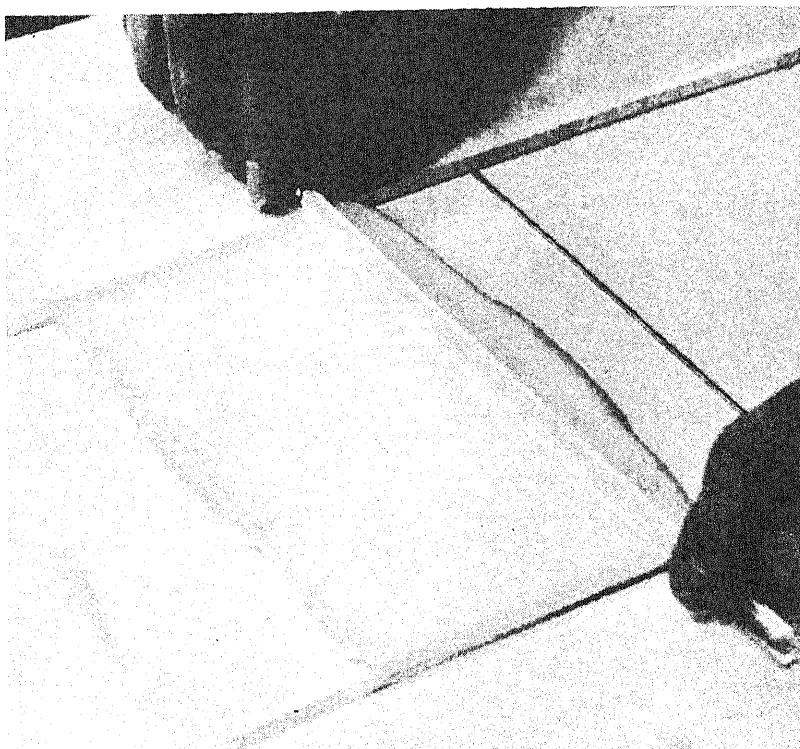
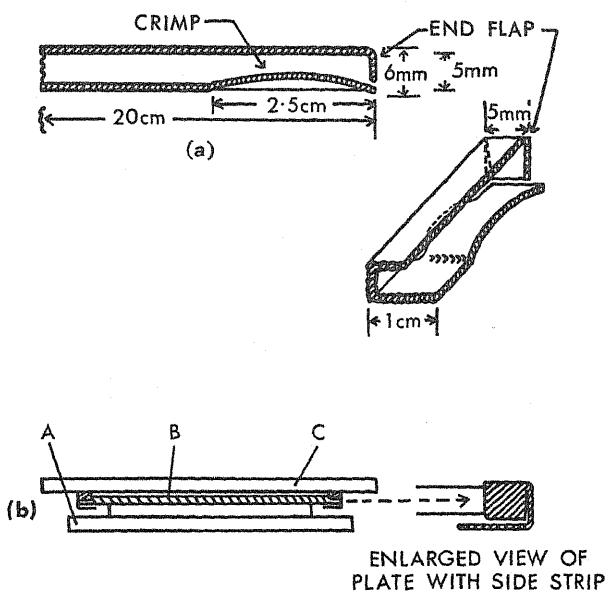


Figure 3.5. Preparation of thin layers using tape and a glass rod. (Reproduced from Lees and DeMuria, *J. Chromatog.*, 8, 108 (1962) through the courtesy of the authors and the Elsevier Publishing Co.)

Peifer²⁹⁶ has developed a complete scheme for chromatography on such small plates. He uses microscope slides for one-dimensional work and lantern slides for two-dimensional work. The slides, cleaned with detergent, water and water-methanol (50:50) acidified with hydrochloric acid, are *dipped*, two at a time, back to back, into a slurry of adsorbent in a nonaqueous medium. The slurry should be well stirred (about 1 min.) before dipping. They are then lifted slowly out of the slurry and allowed to drain on the edge of the container. They dry rapidly, are separated and the edges are wiped clean with tissue. In order to obtain a more durable layer, the plates are exposed briefly to steam to hydrate the plaster of Paris binder. The steam treatment is not actually necessary with silica gel layers. The layers are then



ENLARGED VIEW OF PLATE WITH SIDE STRIP

Figure 3.6. Detail of Duncan side strips, a, and the side strips in use, b. The side strips are fastened to the opposite edges of a glass plate (B) and held in place by the 2.5 cm long indentation. Slurry is poured on the plate and leveled with a glass rod (C) which rests on the side strips. The apparatus is shown on a mounting board (A). The thickness of the metal in the strips determines the layer thickness. (Reproduced from Duncan, *J. Chromatog.*, 8, 37 (1962), through the courtesy of the author and the Elsevier Publishing Co.)



Figure 3.7. A machined spatula for the preparation of thin layers.

activated by suspending them about 6 mm over a hot plate for 1 to 3 min. As mentioned previously, Peifer sometimes added sulfuric acid to his adsorbents to facilitate visualization. The recipes for Peifer slurries are summarized in Table 3.3. The chromatograms containing sulfuric acid are visualized by warming them over a hot plate and the others are sprayed in the normal manner.

TABLE 3.3. RECIPES FOR PEIFER SLURRIES²⁶⁵

| Adsorbent | Slurry Medium | Proportions, g in ml |
|--|--|-----------------------------|
| 1. Silica Gel G | chloroform or chloroform-methanol (2:1, v/v) | 35 g in 100 ml |
| 2. Silica Gel G and sulfuric acid | chloroform-methanol-sulfuric acid (70:30:2.5, v/v/v) | 50 g in 102.5 ml |
| 3. Cellulose powder ¹ | chloroform-methanol (50:50, v/v) | 50 g in 100 ml ¹ |
| 4. Alumina ² | chloroform-methanol (70:30, v/v) | 60 g in 100 ml ² |
| 5. "Florisil" ³ (60-100 mesh) | chloroform-methanol-acetic acid (70:30:1, v/v/v) | 55 g in 101 ml ³ |

¹ 35 g of cellulose powder and 15 g of plaster of Paris are triturated with a minimum volume of methanol. The resulting viscous mixture is then diluted to give the above ratio.

² 45 g of activated alumina and 15 g of plaster of Paris are triturated with a minimum volume of chloroform-methanol and then diluted to the above ratio.

³ 45 g of "Florisil" and 10 g of plaster of Paris are triturated with 1 ml of glacial acetic acid and a minimum volume of chloroform. The result is diluted to the above ratio.

Layers Prepared From Dry Powders

The preparation of layers from dry powders of unbound adsorbents is a slightly different problem. The first method was that of Mottier and Potterat^{267,268} who used the apparatus shown in Figure 3.8. An even simpler affair is illustrated in Figure 3.9. A glass rod equipped with rubber or tape rings to hold it slightly above the plate and a larger ring to keep it centered is rolled over the plate containing an excess of alumina. This latter system is, of course, based upon the Mottier work, but has been more widely used.^{52,71,74,144,146}

Drying of Layers

After the slurry is spread on a plate, it should be allowed to stand for at least 30 min. so that the binder can "set." Indeed it is even recommended by several workers^{46,47,249,319} that more durable layers will result if they are allowed to stand in the air overnight. This would prevent cracking due to "casehardening." For adsorption chromatography, the layers are activated by heating them at 100 to 110° for various periods of time, generally one to several hours. There is little change in the activity when the time is lengthened beyond one hour or the temperature is raised above 110°. For layers containing plaster of Paris, the maximum drying temperature is 128° which is the point

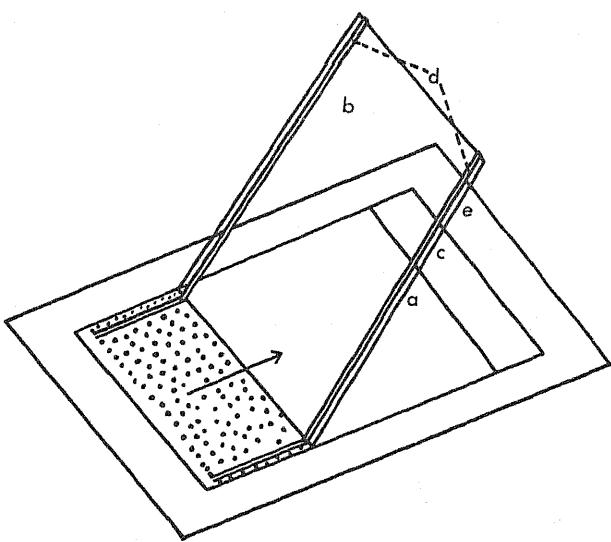


Figure 3.8. Preparation of thin layers of dry, adsorbent powders by the method of Mottier and Potterat. The dry alumina is placed on the glass plate a in front of the moving and leveling guide b. Plate c is a stopping board and d is two rubber bands which determine the layer thickness. Guide b is then moved over the plate. (Reproduced from Mottier, *Mitt. Gebiete Lebensm. Hyg.*, 49, 454 (1958) through the courtesy of the author and the Eidgenoessische Drucksachen-und Materialzentrale, Bern, Switzerland).

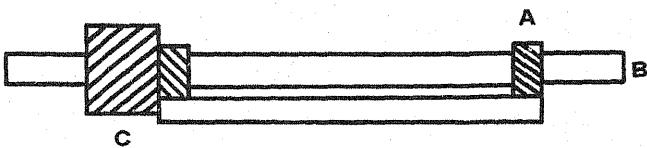


Figure 3.9. End view of a glass-rod leveling device for preparing layers of dry powders. The small spacers (A) are rubber tubing rings placed on a glass rod (B). The large ring (C) centers the gadget. Dry powder is placed on a plate and leveled by rolling the device back and forth.

at which gypsum again dehydrates. For partition chromatography, the layers are normally not activated since the residual water acts as the stationary liquid phase.

Better results with less cracking will be obtained if the layers are

dried in a vertical position as suggested by Desaga. All of the manufacturers of thin-layer equipment sell a metal drying rack which will hold several plates and which can be placed in the drying oven in either a horizontal or vertical position.

The activated plates should be stored in a desiccator or dry box of some type until they are used. The manufacturers of thin-layer equipment provide such containers. However, in the author's laboratory, the plates are carefully stacked in a normal desiccator over calcium chloride or, if large, are kept in a large box containing a beaker of sulfuric acid. After removal from the desiccator, the layers remain active for a time which is inversely proportional to the relative humidity. Normally, plates are active for ten minutes or more, but it is not wise to leave a plate out for several hours during the monitoring of a reaction or the elution of a column.

Other Post-Preparative Operations

The adsorbent sticking to the edges of the plates should be carefully wiped off before using the layers. The layers may then be pre-washed^{388,389} with an appropriate solvent. A 0.5 per cent solution of "Versene" (ethylenediaminetetraacetic acid trisodium salt) was used by Schweiger³⁵⁰ to prewash unbound cellulose layers. Figure 3.10 shows a suggested³⁸⁹ apparatus for carrying out this operation.

The finished plates may, at this stage, be sprayed with a complexing agent such as silver nitrate or boric acid.²⁶⁰ This serves the same purpose as the incorporation of these substances in the slurry as discussed in Chapter 2, but spraying is more convenient in that the plates can be prepared one at a time as needed or in that only a portion of a plate need be treated. Morris²⁶⁰ sprayed with a saturated aqueous solution of silver nitrate or a saturated methanolic solution of boric acid or both. After spraying, the plates were dried at 110° for one hour.

On occasion, it is desirable to use partition chromatography in which the polar, stationary liquid is something other than water. To this end, Teichert, Mutschler and Rochelmeyer^{399,400} have impregnated cellulose layers by dipping them into a 20 per cent (v/v) solution of formamide in acetone. Vaedtke and Gajewska sprayed layers of plaster of Paris bound "Celite" with formamide, propylene glycol

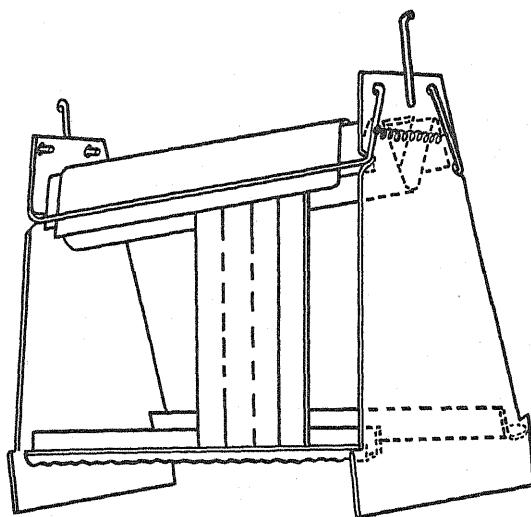


Figure 3.10. An apparatus for prewashing layers. The layers are placed in the apparatus and the washing liquid flows out of a reservoir by means of filter paper and over the layers in a descending manner. (Reproduced from Stanley, Vannier and Gentili, *J. Assoc. Off. Agr. Chemists*, **40**, 282 (1957) through the courtesy of the authors and the Association of Official Agricultural Chemists.)

or paraffin oil.⁴¹⁴ The plates were weighed before and after the spraying to determine the extent of impregnation. Knappe and Peteri²⁰⁰ added polyethylene glycol (15 g to 30 g of Kieselguhr G and 45 ml of water) to the adsorbent slurry before the layers were prepared. In the latter case 0.050 g of sodium diethyldithiocarbamate was also added to prevent peroxide formation in the polyglycol. Such chromatograms are developed with nonpolar solvent systems.

The Preparation of Reversed Phase Chromatograms

The impregnation of thin layers with a high-boiling, nonpolar liquid such as Silicone fluid or a hydrocarbon, leads to a partition chromatogram in which the moving phase is more polar than the stationary phase. Such chromatography is called reversed phase chromatography and normally gives improved separations in a homolo-

gous series of nonpolar compounds. This has been particularly adapted to the fatty acid series.

Malins and Mangold^{230,232} prepared Silicone layers by slowly immersing Silica Gel G layers in a 5 per cent solution of Silicone Oil* in ethyl ether. The operation is carried out at room temperature and both the plates and the liquid must be at this same temperature. In addition, the operation must be carried out slowly. Failure to observe these precautions will produce crumbly layers.

Kaufmann, Makus and Khoë,^{182,186} and Kaufmann and Ko¹⁸¹ have used either a 10 to 15 per cent solution of undecane in petroleum ether (b.p. 40 to 60°) or a 5 per cent solution of tetradecane in the same solvent. Reversed phase layers have been made using paraffin by Winterstein, Studer and Rüegg⁴⁴⁹ (5 per cent in petroleum ether) and by Michalec, Šulc and Měšťan²⁴⁹ (0.5 per cent in ether). All of these were prepared by dipping.

Purdy and Truter³¹⁵ prepared such layers by allowing Silica Gel G layers to be "developed" with a 15 per cent solution of decane in petroleum ether. A similar process was carried out by Wagner, Hörhammer and Dengler using 5 per cent paraffin in ethyl ether.⁴³¹

After the impregnation, the layers are allowed to dry in the air for a few minutes. The development is carried out with polar solvents.

* Dow-Corning 200 fluid viscosity 10 cs. Dow-Corning Corp., Midland, Mich. This preparation is also available from Research Specialities Co. (p. 40) in a spray package for spraying layers.

CHAPTER 4

Application of the Sample

INTRODUCTION

The various factors to be considered in the application of samples to thin layers are the application solvent, the amounts to be applied and the mechanics of the application. These are largely controlled by the type of information or results desired. Thus, for diagnostic or qualitative chromatography a small amount of substance, which need not be accurately known, is applied to a single small spot. This is then developed simultaneously with spots containing possible components of the mixture (reactants, products or known constituents). For quantitative chromatography, a precise amount of the mixture to be analyzed must be applied. For preparative chromatography, large quantities of the mixture to be separated must be applied in a thin uniform band along one edge of a plate.

After the sample has been applied, it can be caused to undergo various reactions which will assist in its identification or will reveal something of its properties.

VARIABLES IN SAMPLE APPLICATION

Application Solvent

The solvent used for the application of samples to thin layers is not particularly critical, but the following factors should be considered. The ideal solvent should boil between 50° and 100° so that it does not evaporate too rapidly but can be easily removed at will. It should completely dissolve the sample to yield at least a one per cent solution, but should be as nonpolar as possible. This allows the

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sample to be concentrated at the center of the spot and not in a ring around the edge. The application solvents should, in any case, be removed as completely as possible before the chromatogram is developed.

Amount of Sample Applied

The easiest and best way to ascertain this quantity is by experimentation with the specific compounds in question. Every compound or mixture should be investigated at several different concentrations. This can be done on a single plate and, simultaneously, information about trace impurities, relative concentrations and separabilities can be obtained. An amount is then chosen which is large enough to be effectively visualized and to show trace impurities, and small enough to give discrete spots with a minimum of tailing. This amount will depend upon the layer thickness and the sensitivity of the visualization procedure which are inversely proportional to each other. Further, it will depend upon the adsorbent and whether the chromatography is adsorption or partition. The former has a much higher capacity. When the sample is too large, tailing³⁰⁷ and high *Rf* values³⁵² will result.

For diagnostic and quantitative chromatography, samples ranging from 10 to 100 γ are normally used with layers about 0.25 mm thick. For preparative work, amounts ranging from 10 to 300 mg can be separated on a single chromatogram depending upon the size of the plate, the thickness of the layers, the ease of separation, and the type of chromatography.

Specific examples of quantitative and preparative thin-layer chromatography will be given in the chapters devoted to these subjects.

MECHANICS OF APPLICATION

The application of samples to bound layers is carried out by touching the tip of a filled capillary, micropipette, or microburette to the adsorbent layer in a manner analogous to that used in paper chromatography. Multiple applications with solvent evaporation after each are usually necessary. The sample is placed or "spotted" about 2 cm or 1 in. from the end of the plate so that the solvent level will be at least 1 cm below the center of the spot. The diameter of the

spot should not exceed 0.5 cm and should be as small as possible. The spot can be kept small by a rapid evaporation of solvent using a stream of dry nitrogen²³² or a low-temperature hot plate.^{223,244,253,329} Honegger¹⁵⁷ and Wagner, Hörhammer and Wolff¹³² advocate the application of the sample in thin bands about 1 cm long rather than the conventional circular spots. The bands are at a 90° angle to the direction of development. Tate and Bishop³⁹⁸ applied samples with a thin wire loop (about 1 mm in diameter) rather than the usual capillary and Metz²⁴⁷ used a paper gadget to make the application point very small.

The manufacturers furnish a "spotting template" to help with the application of a large number of samples. This is a transparent sheet of plastic which fits over a thin-layer plate but does not touch the layer. It is suspended on two side pieces and is marked at 1-cm intervals. The Desaga-Brinkmann model is shown in Figure 4.1.

When a large number of samples must be applied over a long period of time, it is a good idea to cover the layer above the sites of application with a clean glass plate to protect it from moisture.⁴⁶

Diagnostic Chromatography

For diagnostic work, the sample is usually applied through a melting point capillary tube of indeterminate size or a micropipette calibrated in microliters. Such a pipette is furnished by all manufacturers of thin-layer equipment.

Quantitative Chromatography

For this purpose, precise application methods are necessary. A microsyringe* used as such or with a mechanical push-button dispenser (Brinkmann, p. 37) is shown in Figure 4.2. The Agla** micrometer syringe has also been widely used. An appropriate microburette could be used.

Preparative Chromatography

The application of samples for preparative thin-layer chromatography is quite a different problem. Large quantities (50 to 300 mg)

* The Hamilton Company, Inc., Whittier, Calif.

** Burroughs Wellcome Co., The Wellcome Bldg., Euston Rd., London N.W. 1, England.

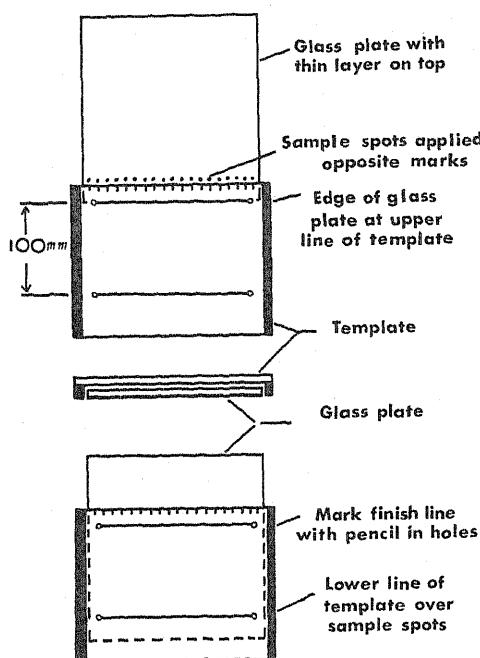


Figure 4.1. The Desaga-Brinkmann "spotting template" in position (Reproduced through the courtesy of Brinkmann Instruments, Inc.)

must be applied in a reasonable time as a uniform band without destroying the layer. Multiple spotting with a single capillary is quite unsatisfactory because it is so tedious and almost always results in a disturbed layer.

The simplest approach to this problem is to blow a fine jet of sample and solvent into the layer from a suitable pipette while moving the pipette in a uniform motion over the plate. The pipette should have a small orifice and the operator needs a deft pair of hands.* Honegger¹⁵⁸ has suggested that the sample be applied in a shallow V-shaped ditch made on the starting line. Care must be taken so that the ditch does not go through to the glass plate.

In addition to these methods, two mechanical gadgets are commer-

* The author has seen this done by Mr. P. J. DeMuria at the Chas. Pfizer Laboratory in Groton, Conn. and it has been reported elsewhere.

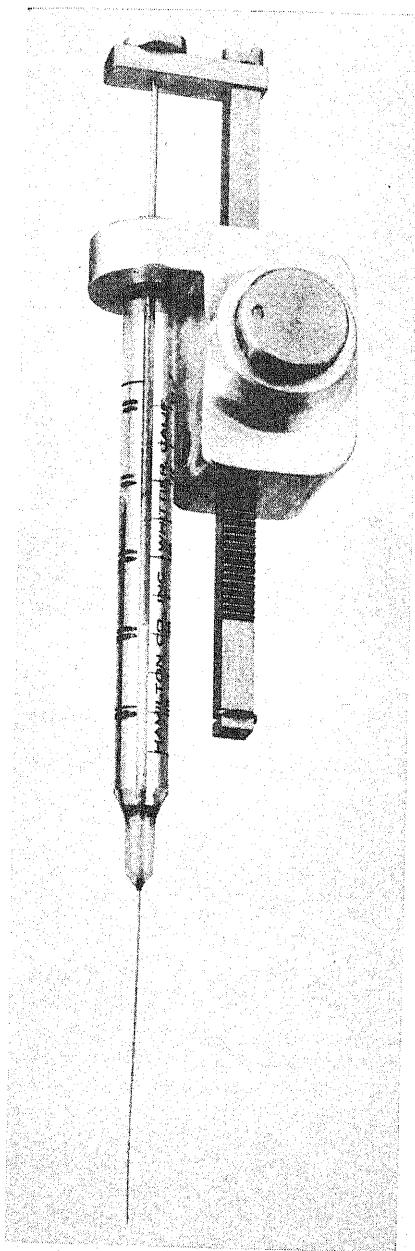


Figure 4.2. Microsyringe with push button dispenser for use in quantitative thin-layer chromatography. (Reproduced through the courtesy of Brinkmann Instruments, Inc.)

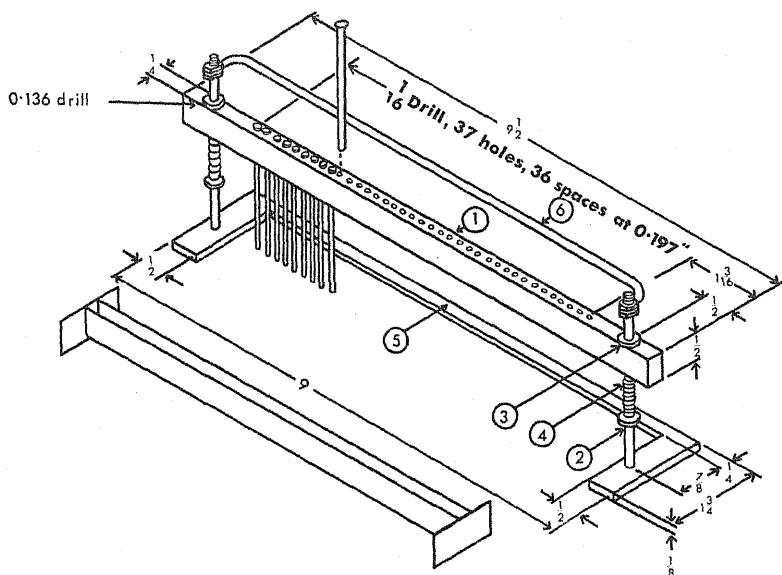
Thin-Layer Chromatography

Figure 4.3. The Morgan apparatus for sample application in preparative thin-layer chromatography. The apparatus consists of an aluminum bar (1) with holes drilled near its ends which permit it to slide freely on two vertical $\frac{1}{8}$ inch brass rods (2) and is held up against adjustable stop washers (3) by partially compressed springs (4). The top and bottom stop washers are $\frac{1}{4}$ inch OD and $\frac{3}{32}$ inch ID and are enlarged slightly with a punch so that they may be forced onto the vertical rods. The bottom washers which support the springs are soldered in place while the top washers remain in place by friction and may be adjusted by forcing either up or down. The two vertical rods threaded, screwed into tapped holes, and soldered in place in the cutout base plate (5) and the upper horizontal tie rod (6) form the rigid support for the apparatus. Thirty-seven precisely drilled $\frac{1}{16}$ inch holes spaced 0.197 inch (0.5 cm) on centers in the aluminum crossbar carry the spotting pipettes. These are made from pieces of 1.5 mm OD Pyrex melting point capillary tubing (Corning #9530) selected so that they will just pass through the holes in the crossbar with no friction. A funnel stop is blown at the upper end of each tube, the tubes cut at a length such that the tips will extend to within $\frac{1}{16}$ inch of the surface of the chromatographic plate when placed in the crossbar. The delivery tips are fire polished and squared off by grinding on a flat piece of fine carborundum wetted with water. Progress in the latter operation should be observed under suitable magnification and the grinding continued only until the contact surface of the tip is smooth.

A sample trough of suitable volume and design to be used with the apparatus in spotting preparatory plates may be constructed from metal, glass, or plastic depending upon the chemical nature of the samples and solvents employed. A trough of the design shown was constructed of No. 23 gauge Monel metal and has proven to be satisfactory for use with non-corrosive solvents. (Reproduced from Morgan, *J. Chromatog.*, 9, 379 (1962) through the courtesy of the author and the Elsevier Publishing Co.)

cially available for sample application in preparative chromatography. These are the multiple capillary arrangement of Morgan²⁵⁸ and the moving syringe system of Ritter and Meyer.³²⁹

The Morgan Apparatus. The Morgan apparatus is, simply, some thirty-seven capillary tubes arranged in a movable holder and is shown in Figure 4.3 with instructions for its construction. All of the tubes are filled by dipping them into a trough containing the sample in solution and they spot simultaneously when touched to a thin layer. It has been used with great success by the author and elsewhere at the University of Connecticut, and is commercially available from the A. H. Thomas Co.*

The Ritter and Meyer Apparatus. The Ritter and Meyer apparatus is a syringe equipped with a pressure valve that is supported by two bars on which it slides freely. The sample is placed in the syringe, a slight pressure is applied and the syringe is moved back and forth spraying a thin jet of sample. The plate holding the layer is placed on an asbestos-covered aluminum block which is heated to facilitate the removal of solvent. The apparatus is shown in Figure 4.4. It is commercially available from Desaga-Brinkmann (see p. 37).

Sample Application to Unbound Layers

The unbound layers (excluding the Woelm and Anisil special adsorbents) are not mechanically stable enough to touch so that samples are applied in small drops from a short distance away.⁵² When unbound layers are developed in a near horizontal position, the sample can be adsorbed on a small amount of adsorbent and placed on the thin layer as such.²⁶⁰ This latter technique is especially useful if one wishes to rechromatograph a sample on a new layer. The adsorbent containing the sample can simply be scraped off of one plate and applied to another without elution. Samples are applied to Woelm and Anisil layers in the same manner as with bound adsorbents.

CHEMICAL REACTIONS ON APPLIED SAMPLES

Sometimes, it is advantageous to modify or prepare a derivative of a sample after it has been spotted on a thin layer. This can be accom-

* Arthur H. Thomas Co., Vine Street at 3rd, Philadelphia, Pa.

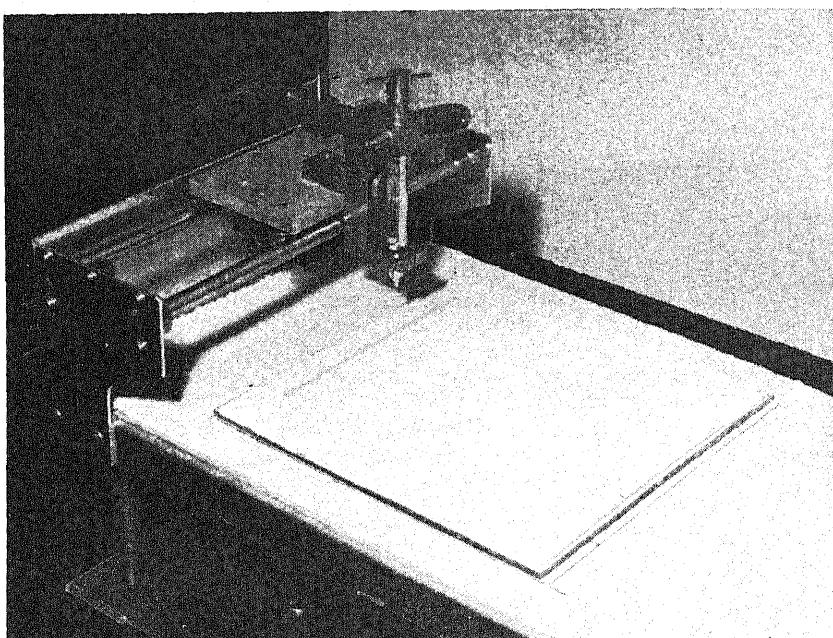


Figure 4.4. The Ritter and Meyer apparatus for sample application in preparative thin-layer chromatography. (Reproduced from Ritter and Meyer, *Nature*, 193, 941 (1962) through the courtesy of the authors and Macmillan and Co., Ltd.)

plished by adding the reagent to the developing solvent. Thus, bases can be regenerated from their salts and compounds can be brominated or oxidized by putting the appropriate reagents in the developing system. Alternately, a drop of reagent can be added to the spotted sample before chromatography. This technique has been used for oxidation, dehydration, hydrogenation and the preparation of derivatives. These reactions are carried out in the following manner.

Regeneration of Bases

The amine or alkaloid is spotted on the layer as its hydrochloride, and a base, diethylamine⁴³⁶ or ammonia,⁴³² is added to the developing solution to the extent of 1 or 2 per cent. A similar reaction was performed in the thin-layer separation of the halide ions⁴⁵⁷ by Seiler and Kaffenberger. The halides were spotted as their sodium and po-

tassium salts, but the developing solution contained 10 per cent of concentrated ammonium hydroxide and the ions migrated as their ammonium salts. Mottier²⁶⁶ neutralized dye solutions before chromatography on alumina by placing a drop of 1 N sodium hydroxide solution on the layer, drying it and then spotting the sample.

Bromination¹⁸⁶

The sample is spotted in the normal way and the chromatogram is developed by solvents containing 0.5 per cent (by volume) of bromine.

Oxidation

Oxidation is brought about by addition of an oxidizing agent to the developing system or by direct application over the sample spot. Thus, Mangold^{230,231,232} separated saturated and unsaturated fatty acids on reversed phase layers with a mixture of acetic acid-peracetic acid-water (15/2/3). The unsaturated compounds were oxidized and moved to the solvent front whereas the saturated compounds were separated in a normal manner.

In an alternate technique,²⁵² the sample to be oxidized is spotted in the normal way and covered with a drop of glacial acetic acid, saturated with chromic anhydride. The chromatogram is then developed with solvents which are not easily oxidized.

Dehydration²⁵²

The sample, spotted as usual, is covered with a drop of concentrated sulfuric acid and developed with solvents such as hexane which are resistant to sulfuric acid.

Phenylhydrazone Formation²⁵²

The sample, spotted as usual, is covered with a drop of phenylhydrazine and then developed with a noncarbonyl solvent.

Semicarbazone Formation²⁵²

A 10 per cent solution of semicarbazide hydrochloride is neutralized with sodium hydroxide and spotted with the sample. The chromatogram is developed with a noncarbonyl solvent.

Hydrogenation¹⁸⁶

The spotting region of a layer is sprayed with a colloidal solution containing 1 to 2 per cent palladium in an electrolyte free solution.* The plate is dried in an oven, the sample is spotted in the region containing the palladium, and the layer is exposed to a hydrogen atmosphere in a vacuum desiccator. The plate is developed in the normal manner.

Irradiation³⁷¹

The spotted sample is irradiated with a suitable energy source in the desired atmosphere and developed in the normal manner.

Salt regeneration and bromination normally go to completion but the other reaction products are contaminated with starting materials which can be used, untreated, on the chromatograms as standards. The reactions listed certainly represent only a small portion of the possibilities for this technique.

* The solution is commercially available from Firma Dr. Th. Schuchardt, Ainmillerstr. 25, München 18, Germany.

CHAPTER 5

Choice of a Solvent System

INTRODUCTION

The type of solvent system chosen to make a given separation will depend primarily upon whether the chromatography is adsorption or partition. Adsorption chromatography is the simpler of the two because the solvents are completely miscible in one another and their ability to move a sample follows the normal eluotropic series. On the other hand, partition chromatography involves a selection of a two-phase system which will have desirable partition coefficients for the particular sample to be separated. Furthermore, partition chromatography can be of two types depending upon whether the stationary phase is polar (normal) or nonpolar (reversed phase). An even more complex situation involving various ionic species arises with ion-exchange chromatography on thin layers.

It is sometimes advantageous to add a small amount of some special reagent to developing systems to bring about a desired chemical reaction, to improve separations or to change the layer in some manner.

ADSORPTION CHROMATOGRAPHY

The normal worker will spend more time searching the literature for solvent systems to use in adsorption chromatography than he will in determining these systems by experimentation. This is particularly true since the exact adsorptive properties of layers tend to vary from one laboratory to another. The classic eluotropic series of Trappe,⁴⁰³ Strain³⁹³ and Knight and Groennings²⁰² apply to thin-layer chroma-

tography²³² and are given in Table 5.1. The more polar solvents producing the greatest migrations are at the bottom of the columns.

A preliminary study can be made in two ways. The first, devised by Izmailov and Shraiberg,¹⁶⁹ Crowe,⁶³ and further developed by Stahl,^{366,369,370} is the simpler. It involves the spotting of the sample in several places, an inch or so apart, on a single layer. Pure solvents of various polarities are deposited at the centers of these spots by means of a capillary and the outer edge of the solvent spot is noted. The layer is sprayed or visualized in some manner and the solvent which moves the sample about halfway between the origin and the outer edge is the best solvent. The second way to make a preliminary study is by running chromatograms with various solvents. In some laboratories, a series of chromatography jars containing certain key solvents like hexane, benzene, ether and methanol are kept ready for this purpose. In one of these ways, the ideal pure solvent or a pair of solvents whose properties "bracket" the material to be separated (one moving it less and one moving it more than desirable) are selected. In the latter case, various mixtures are investigated, or a gradient elution

TABLE 5.1. ELUOTROPIC SERIES OF SOLVENTS

| Trappe ⁴⁰³ | Strain ³⁹³ | Knight and Groennings ²⁰² |
|-----------------------|-----------------------------|--------------------------------------|
| Light petroleum | light petroleum 30-50° | heptane |
| Cyclohexane | light petroleum 50-70° | diisobutylene |
| Carbon tetrachloride | light petroleum 50-100° | benzene |
| Trichloroethylene | carbon tetrachloride | isopropyl chloride |
| Toluene | cyclohexane | isopropyl ether |
| Benzene | carbon disulfide | ethyl ether |
| Dichloromethane | anhydrous ethyl ether | ethyl acetate |
| Chloroform | anhydrous acetone | sec-butyl alcohol |
| Ethyl ether | benzene | ethyl alcohol |
| Ethyl acetate | toluene | water |
| Acetone | esters of organic acids | acetone |
| n-Propanol | 1,2-dichloroethane | methanol |
| Ethanol | alcohols | pyruvic acid |
| Methanol | water | |
| | pyridine | |
| | organic acids | |
| | mixtures of acids or bases, | |
| | water, alcohols, or pyr- | |
| | idine | |

system³³⁴ can be used. The gradient elution technique will be discussed more extensively in Chapter 6.

The author and others^{144,232} strongly recommend that developing mixtures be kept as simple as possible. In most situations, a mixture of two components will produce satisfactory results. For example, the author has separated a wide range of compounds such as glycosides, aglycones and nitrogen heterocyclics with mixtures of methanol and ether.

One should not become too concerned about whether the substance to be chromatographed is soluble in the solvent system. There appears to be no direct relation between *Rf* and solubility. This is probably because migration involves solution from a molecular or amorphous state while normal solubility requires the breakup of a crystal structure. Thus, sucrose and glucose can be moved readily on silica gel by methanol-ether solutions in which they are apparently insoluble.

NORMAL PARTITION CHROMATOGRAPHY

In this case, the substances to be separated (generally very polar compounds like sugars or amino acids) are caused to distribute themselves between a polar stationary phase (water, acetic acid, phenol or formamide) on the layer and a moving nonpolar phase. The nonpolar phase normally consists of two organic liquids having different polarities. Since the actual developing solution must contain a miscible mixture of all three of these components, it is likely that the literature will provide easier information than random experimentation.

The two liquids constituting the nonpolar phase provide the major point of variation for partition chromatography. Within the bounds of mutual solubility, their ratio can be varied to provide a greater or lesser dissolving power for the moving phase. The literature on paper chromatography provides at least a starting point in search for a system and is directly applicable when the layer consists of powdered cellulose³¹⁹ or Silica Gel G.⁴⁴ The literature summarized in Chapter 11 cites many solvent systems which have been used in thin-layer work.

REVERSED-PHASE PARTITION CHROMATOGRAPHY

In this case, the stationary phase is nonpolar and is usually a high boiling hydrocarbon or a Silicone liquid which has been applied to the layers are described in Chapter 3, p. 49. The moving, more polar phase, might be water, acetic acid, acetonitrile, alcohol, or some combination of these solvents. The method is useful for separating compounds of a homologous series such as the fatty acids or their derivatives. Table 5.2, taken mainly from Mangold²³² suggests several possible solvent systems.

TABLE 5.2. DEVELOPING SYSTEMS FOR REVERSED PHASE PARTITION CHROMATOGRAPHY*

| Solvents ¹ | Ratios, v/v | Stationary Phases ² | Lipid Classes Fractionated | Ref. |
|--|-------------|--------------------------------|--|------|
| Methanol | — | higher paraffins | carotenals | 449 |
| Acetic acid-water | 24/1 | undecane | fatty acids | 182 |
| — | 3/1 | Silicone oil | fatty acids and their methyl esters | 230 |
| — | 17/3 | Silicone oil | fatty acids and their methyl esters and aldehydic cores derived from lecithins | 230 |
| Acetic acid-acetonitrile | 1/1 | undecane | fatty acids | 182 |
| Acetic acid-acetonitrile-water | 2/14/5 | Silicone oil and squalane | methyl-esters of fatty acids | 230 |
| Methyl ethyl ketone-acetonitrile | 7/3 | higher paraffins | cholesteryl esters of fatty acids | 184 |
| Chloroform-methanol-water | 5/15/1 | undecane | diglycerides | 182 |
| Acetone-acetonitrile | 7/3 | undecane | triglycerides | 182 |
| Acetone-ethanol-water | 6/1/3 | Polyethylene | methyl esters of fatty acids | 232 |
| Acetic acid-formic acid-water ³ | 2/2/1 | Silicone oil | fatty acids saturated/unsaturated | 230 |
| Acetic acid-peracetic acid-water | 15/2/3 | Silicone oil | fatty acids saturated/unsaturated | 230 |

¹ All solvent mixtures must be saturated with the stationary phase.

² Silica Gel G served as a carrier for the stationary phases. Only polyethylene was applied directly to the plates.

³ Chromatographed at 4-6°. (All others at room temperature.)

* Reproduced from Mangold, *J. Am. Oil Chemists' Soc.*, **38**, 708 (1961) through the courtesy of the author and The American Oil Chemists' Society.

ION-EXCHANGE CHROMATOGRAPHY

The modified-cellulose ion exchangers DEAE and ECTEOLACelluloses have been used by Randerath^{316-318,320} for the separation of nucleotides. He used dilute hydrochloric acid (0.02 to 0.04 N) to develop his chromatograms. Determann, Wieland and Lüben⁸² used a gradient elution system, adding increasing amounts of sodium chloride to a phosphate buffer (*pH* 7.2), to separate enzymes and nucleotides on "Sephadex." Berger, Meyniel and Petit²⁹ used 1 M sodium nitrate to develop halogen ions on "Dowex 1" and acetic acid-methanol-acetone to separate fluorescein dyes on the same resin.

MISCELLANEOUS SPECIAL ADDITIVES

Several substances have been added to developing solutions for one reason or another. As mentioned (p. 58), bases,^{332,436} bromine^{186,295} and oxidizing agents²³⁰⁻²³² are added to bring about some reaction with the sample or some part of it. Seiler and Seiler³⁵⁹ have added small amounts (0.4 per cent) of the complexing agent, 2,5-hexanedi-one, to facilitate the separation of inorganic cations. Mistryukov²³⁴ added a small amount of decalin to developing solutions to be used on nonbound layers. The decalin increased the mechanical stability of the layer so that it could be visualized by spraying.

Small amounts of acidic or basic solvents are often added to developing systems to facilitate the separation of acids and bases, respectively, and to prevent "tailing" or smearing. "Tailing" is due, among other things, to the presence of more than one ionic species of the substances being chromatographed.²⁷⁴ Thus, Mangold²³² and others²⁶⁴ suggest the addition of 1 to 2 per cent of acetic acid to systems for developing chromatograms of fatty acids. In a similar manner, systems containing ammonia^{232,256} and diethylamine^{332,436} are used for separating nitrogenous lipids and other bases. These additives buffer the system and keep the material being separated exclusively in the non-ionic form.

CHAPTER 6

Development of Thin-Layer Chromatograms

INTRODUCTION

The development process, that is, the actual passing of solvents through thin layers to cause separations, is a major point of variation in thin-layer chromatography. Like the preparation of layers, it challenges the imagination of the laboratory worker and many ingenious techniques have resulted.

Thin-layer chromatography has been carried out in an ascending manner on vertical or near horizontal plates, in a descending manner on vertical plates, or in a linear or circular manner on horizontal plates. The development can be performed discontinuously, one pass or multiple pass, in a stepwise fashion with different solvents, continuously or by gradient elution methods. The layers can be scored in such a way that the chromatography is carried out in wedge or modified wedge-shaped areas. The process can be carried out in a two-dimensional fashion with different solvent systems being used in each direction. The layer or the sample may also be modified between two developments. These techniques will be discussed in detail in this chapter.

Finally, the various factors which influence the development or, more precisely, the R_f values resulting therefrom will be considered. These include the nature of the adsorbent and of the developing system, the nature and amount of the sample, the chamber saturation, the use of multicomponent solvent systems and the temperature. Some

methods of reporting *Rf* values and the causes and alleviation of tailing will be briefly discussed.

METHODS OF DEVELOPMENT

Ascending Chromatography

Most thin-layer chromatograms are developed by placing a glass plate, containing the sample on a bound, thin layer, in a vertical position in a closed, saturated system such that the bottom of the layer dips into the developing solvent. After the solvent ascends 10 to 15 cm, the plate is removed, dried, and visualized in some manner. Two or more layers can normally be processed in the same container provided that care is taken to completely saturate the system.

Kirchner, Miller and Keller¹⁹³ found that when water is a component of the developing system, the adsorbent layer tends to slide off of the plate below the solvent level. This problem was solved by standing the plate containing the layer on a cotton wad saturated with solvent. The author has observed a similar effect with very polar solvent systems, but found that even though the adsorbent below the liquid surface slides off, enough remains to carry the solvent into the layer satisfactorily.

While the plates are normally leaned against the side of the chromatographic jar or tank, several pieces of apparatus have been devised for holding them. A stainless-steel rack which will hold two large plates (20×20 cm) is available from Desaga-Brinkmann (p. 37), but a more versatile apparatus is available from Kensington Scientific Corp. (p. 36). This apparatus, pictured in Figure 6.1, is constructed from glass rods and will hold two plates (10×20 cm). It is suspended in a chromatographic chamber such that it can be raised or lowered without opening the chamber and thus allows the equilibration of a layer with the solvent before actual development. Such an equilibration step is recommended for partition chromatography.

When unbound layers are used (p. 46) they must be kept in a near-horizontal position (10 to 20° from horizontal) so that the adsorbent does not slide off. While other schemes have been devised,²⁵⁴ the simplest method for supporting these plates is that of Mottier and Potterat.²⁶⁹ As shown in Figure 6.2, the plates are propped in a large

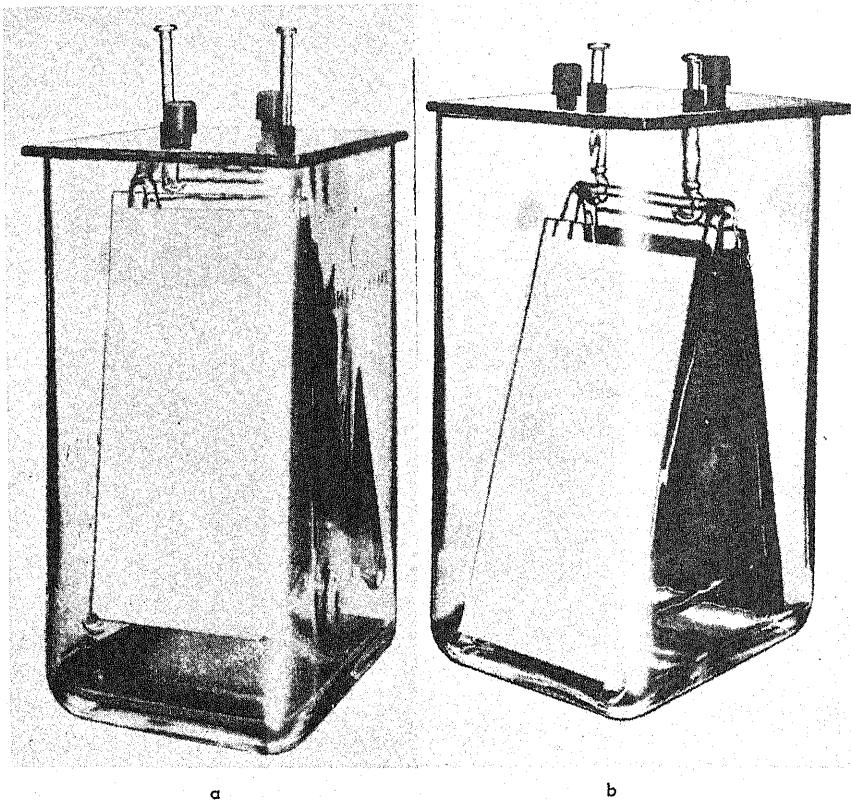


Figure 6.1. Chromatographic chamber containing a movable plate holding device which can be used to equilibrate thin-layer chromatograms prior to development. It is shown in a raised position, a, and a lowered position, b. (Reproduced through the courtesy of the Kensington Scientific Corp.)

Petri dish or crystallizing dish and the dish is tilted so that the solvent is confined to the portion touching the layer.

Descending Chromatography

The technique of descending thin-layer chromatography has essentially been worked out and applied by W. L. Stanley and his co-workers.^{383-385,388} They suspended 0.5 in. wide "chromatostrips"¹⁹³ coated with a silicic acid starch layer in an especially designed chamber as shown in Figure 6.3. The developing solvent is carried from the reservoir by a piece of coarse filter paper which is clipped to the top

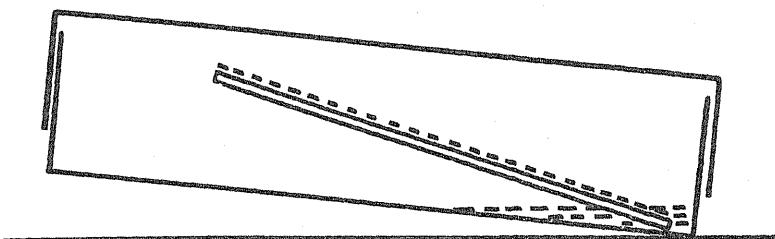


Figure 6.2. Development of a thin layer of unbound adsorbent. (Reproduced from Mottier and Potterat, *Anal. Chim. Acta*, 13, 46 (1955) through the courtesy of the authors and the Elsevier Publishing Co.)

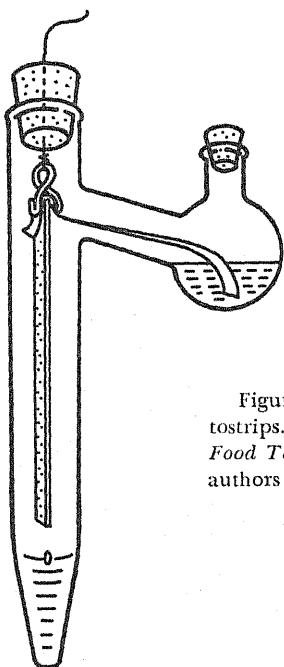


Figure 6.3. Descending chromatography on "chromatostrips." (Reproduced from Stanley, Ikeda and Cook, *Food Technol.*, 15, 381 (1961) through the courtesy of the authors and the Institute of Food Technologists.)

end of the layer. Such a system is advantageous in that it permits a continuous chromatography from which samples of eluent can be removed for analysis.³⁸⁵ Furthermore, it allows the use of relatively nonpolar solvents over a long time period and gives better separations.

Descending chromatography on wide plates containing the somewhat more fragile Silica Gel G layers has been carried out by Birkofe^r, Kaiser, Meyer-Stoll and Suppan³⁶ and by Zöllner and Wolfram.⁴⁵⁸ The apparatus of the latter authors is much the simpler of the two and is shown in Figure 6.4. The solvent is carried to the surface of the layer (facing down) by a piece of paper which also helps to saturate the atmosphere. The apparatus has an additional advantage in that a paper chromatography jar can easily be modified for the purpose.

Mistryukov²⁵⁵ has described an apparatus for the development, by descending chromatography, of unbound layers.

Horizontal Chromatography

Linear. Mistryukov²⁵⁴ has published a method for carrying out linear chromatography on completely horizontal unbound layers and the continuous chromatography of Brenner and Niederwieser^{45,46} as described below is on horizontal layers.

Circular. The original publication on bound layers by Meinhard and Hall²⁴⁶ involved circular chromatography of inorganic ions and since then, the technique has been used extensively.^{51,210,211,246,306,313,368} The sample is placed at the center of a layer and the solvent is fed into the center of the spot with a pipette^{210,211,246} or a cotton wick⁵¹ held in contact with the layer. The resulting chromatogram consists

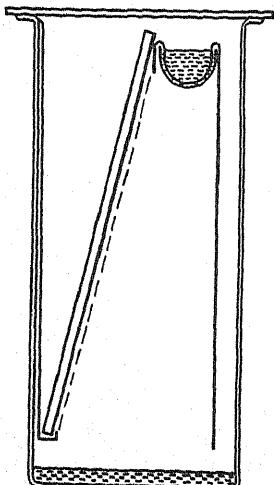


Figure 6.4. Descending chromatography on wide plates. The dotted line represents the layer. (Reproduced from Zöllner and Wolfram, *Klin. Wochschr.*, 40, 1098 (1962) through the courtesy of the authors and Springer Verlag).

of concentric rings. An alternate method which allows the comparison of several samples involves the placement of samples around and equidistant from a center point. Solvent is fed into the center point and the result consists of a series of chromatograms radiating from the center point. Figure 6.5 shows one arrangement for circular chromatography by Stahl.³⁶⁸ The apparatus requires a plate with a small hole in it.

Discontinuous Development

Single Pass. The vast majority of thin-layer chromatography has been carried out by allowing a single solvent system to pass through the layer one time. As discussed below, however, there is some reason to believe that better results could be obtained by different techniques.

Multiple Pass. Under the proper conditions, the development of a layer several times with the same solvent (with drying between developments) offers considerable advantage. The technique has been extensively used^{158,179,222,269,380,398} and has, in itself, been the subject of two important papers.

The first paper¹⁷⁵ dealt with paper chromatography, but should be equally applicable to thin layers. In it, Jeanes, Wise and Dimler state that the optimum number of passes, n_{\max} , can be predicted using Equation (6.1) where Rf_1 and Rf_2 are the values of individual com-

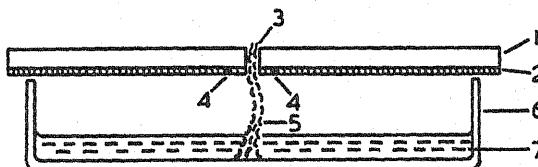


Figure 6.5. Circular thin-layer chromatography.

- | | |
|------------------------------------|----------------------|
| 1 glass plate (20×20 cm) | 5 cotton wick |
| 2 Silica Gel G layer (0.25) | 6 Petri dish cover |
| 3 2 mm hole | 7 developing solvent |
| 4 sample spots | |

(Reproduced from Stahl, *Parfüm. Kosmetik*, 39, 564 (1958) through the courtesy of the author and Dr. Alfred Huethig Verlag, Heidelberg, Germany).

$$n_{\max} = \frac{\log Rf_1 - \log Rf_2}{\log (1 - Rf_2) - \log (1 - Rf_1)} \quad (6.1)$$

pounds or Equation (6.2) where R_f is the average R_f value of the

$$n_{\max} = \frac{1}{R_f} - 1 \quad (6.2)$$

compounds. In the second paper¹⁰¹ which does discuss thin-layer chromatography, Thoma gives Equation (6.3) for the calculation (using the same symbols as 6.1 and 6.2). It is apparent from these equations

$$n_{\max} = \frac{-1}{\ln_e (1 - R_f)} \quad (6.3)$$

that systems with low R_f values (0.2 or less) can be greatly assisted by repeated developments while those systems with high R_f values cannot. Thoma was able to show that better separations were, in fact, obtained by several developments with a less polar solvent system than by one development with a more polar one.

Stepwise Development. Stahl and his co-workers^{309,378} devised a technique in which the layer was developed twice to differing heights with different solvent systems. Such a system is ideal when the sample contains a group of similar polar molecules and a group of similar nonpolar molecules. The chromatogram is first developed halfway (about 6 to 7 cm) with a polar system which deposits the nonpolar compounds at the solvent front about halfway up the layer and resolves the polar mixture in the bottom half of the chromatogram. The chromatogram is removed, dried and developed with a nonpolar solvent system. This system passes over the separated polar substances and resolves the nonpolar mixture in the top half of the chromatogram. This method is illustrated in Figure 6.6³⁷⁸ by the separation of a mixture of glycosides (polar) and aglycones (nonpolar). Similar schemes have been used by Billeter and Martius³⁵ for the separation of the K vitamins and by Mangold and Kammerer²³³ for the separation of lipid derivatives.

Weicker⁴³⁸ used still a third step for the separation of complex lipid mixtures. He first developed silica gel layers to 3 cm with propanol-ammonia (2:1). This carried the fatty acids, cholesterol and cholesterol esters to the solvent front and resolved lecithins and polar

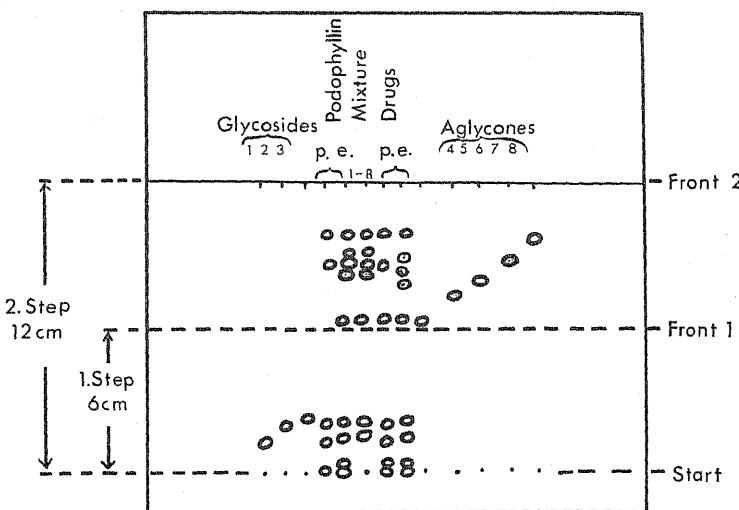


Figure 6.6. Thin-layer chromatography of *Podophyllum* components using a stepwise technique. 1. α -Peltatin- β -D-glucoside; 2. Podophyllotoxin glucoside; 3. β -Peltatin- β -D-glucoside; 4. 4'-Demethylpodophyllotoxin; 5. α -Peltatin; 6. Podophyllotoxin; 7. β -Peltatin; 8. 1-Deshydroxypodophyllotoxin. The plant extracts (marked podophyllin) and the drugs of two species of *Podophyllum*, *peltatum* L. (p) and *P. emodi* Wall (e) are shown along with a synthetic mixture of the eight compounds. The adsorbent was Silica Gel G and the solvent systems for steps 1 and 2 were 10% methanol in chloroform and 35% acetone in chloroform respectively. (Reproduced from Stahl and Kaltenbach, *J. Chromatog.*, 5, 548 (1961) through the courtesy of the authors and the Elsevier Publishing Co.)

lipids. A second development with chloroform-benzene (3:1) to 10 cm resolved the fatty acids and free cholesterol and carried the cholesterol esters to the solvent front. The layer was then rotated 180° and developed to a height of 4 cm with carbon tetrachloride to resolve cholesterol esters.

Still another variation⁴¹³ involves replacement of old adsorbent with new, and subsequent chromatography. Thus, in the separation of lipids, the crude mixture is developed first with ether-petroleum ether (5:95) on an unbound alumina layer. This moves the triglyceride fraction and the cholesterol esters. The adsorbent containing these components is removed and they are then assayed. The portion of the plate which contained these components is then cleaned

and a new layer added. Development with petroleum ether-ether-acetic acid (94.5:5:0.5), then moves and separates cholesterol, fatty acids and phospholipids on the new layer and they are subsequently removed and assayed.

Gradient Elution. Rybicka^{334,335} and Determann, Wieland, and Lüben⁸² have developed apparatus for gradient elution of thin-layer chromatograms, that is, for changing the solvent during the actual course of development. In this case a chromatography jar equipped with a magnetic stirrer is used. The chromatogram is dipped into one solvent (the less polar) in the bottom of the jar. Simultaneously, a second solvent (the more polar) is added from a burette which is connected to a glass tube extending through a hole in the top of the jar to the solvent surface. As the development and the addition continue, the properties of the solvent system slowly approach those of the added solvent. Using this technique, Rybicka separated glycerides^{334,335} on silica gel layers, Hofmann¹⁵³ separated proteins on hydroxylapatite and Determann, Wieland and Lüben⁸² separated enzymes and nucleotides on "DEAE-Sephadex."

Chromatography in Shaped Areas. Sometimes, layers are scored or divided with a sharp object into various shaped areas such as is shown in Figure 6.7. These shapes are such that the solvent enters through a narrow area of the layer and is forced to expand into the wider areas. The method was introduced by Mottier²⁶⁶ who used shape 6.7a in unbound alumina layers. Shapes 6.7b and 6.7c have been used by Stahl³⁶⁸ and Peereboom and Beekes²⁹⁵ and a wedge shape, 6.7d, is preferred by Prey, Berbalk and Kausz.³⁰⁷ The result of such a chromatogram is that the horizontal dimension of the spots is increased and the vertical dimension is decreased. The resulting narrow bands allow a greater number of compounds to be more completely separated than usual. An additional advantage of this technique is that small quantities of one component can be found in the presence of a large amount of another.³⁰⁷ The chromatogram 6.7c is the result of the separation of a mixture of one hundred parts of saccharose to one part of raffinose.

Two-Dimensional Chromatography. Sometimes, more components are in a mixture than will separate clearly on a one-dimensional strip. Or, perhaps, the mixture contains substances that differ from one

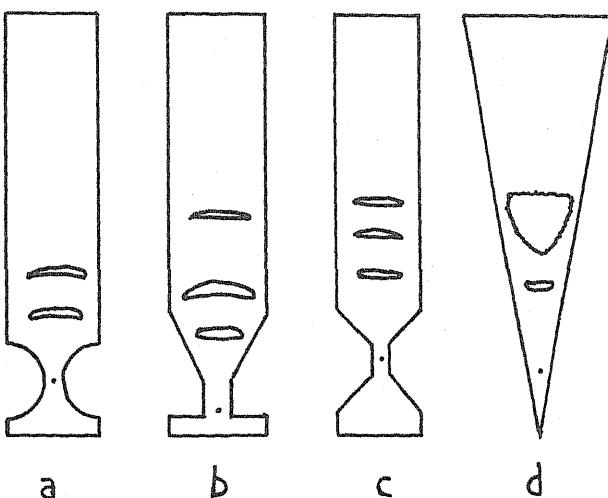


Figure 6.7. Chromatography in shaped areas according to Mottier,²⁰⁰ a, Stahl,²⁰¹ b, Peereboom and Beekes,²⁰⁵ c, and Prey, Berbalk and Kausz.²⁰⁷ d.

another so much that two solvent systems should be used. In these situations chromatography is often carried out in two directions which are perpendicular to one another. The general procedure is as follows. The sample is spotted in one corner of a square layer and developed so that the components are resolved along one edge of the plate. The chromatogram is then removed from the developing jar, dried and placed in a second solvent so that the previously formed component spots along the bottom of the plate are again submitted to chromatography. Such a system has been used extensively in thin-layer chromatography.^{21,44,46,87,182,184,193,221,269,317,352,371} The major point of variation in this technique is what is done to the solvent system or to the layer between the two developments.

Normally, two different solvent systems are used in the two developments so that the sample is exposed to the broadest possible conditions. The layer can be modified between the developments by dipping it in a hydrocarbon or a Silicone oil solution²³² to make a reversed phase chromatogram. The latter system has been especially useful in the lipid field where adsorption chromatography is used in one direction to separate the lipid classes and reversed phase chro-

matography is used in the second direction to resolve the classes. Anet⁵ has actually changed the adsorbent between developments. He first chromatographed 2,4-dinitrophenylhydrazones on Silica Gel G in one direction with toluene-ethyl acetate (3:1); then scraped off the adsorbent above the row of spots and replaced it with Aluminum Oxide G, and finally developed with the same solvent in the new adsorbent. The layers were made in each case with the modified⁴⁵² Camag apparatus (see p. 36).

Any of the modifying reactions listed in Chapter 3 (p. 57) can be carried out after the first development. Thus, hydrogenation,¹⁸⁶ bromination¹⁸⁶ or irradiation³⁷¹ sometimes modifies a mixture in such a way that the compounds which did not separate in the first development will do so in the second. This technique, called separation-reaction-separation by Stahl,³⁷¹ is clearly shown in the chromatogram in Figure 6.8 prepared by Stahl, where an irradiation reaction was carried out between separations. The chromatogram also shows the use of standards.

Continuous Development

Continuous development, or elution through a thin layer of adsorbent, offers two advantages. First, samples can be eluted off the end of the chromatogram, thus allowing the separation of mixtures containing components of widely differing polarities. Second, it allows the use of a relatively nonpolar solvent system over a long period of time. In this respect, it should be noted that a very polar developer tends, not only to move things too fast, but to override small differences between mixture components. Of course, with increased developing times, the spots diffuse more and separations become less sharp.

One method which has been used for this purpose (p. 68) is descending chromatography.^{385,388} In this technique, the solvent drips from the bottom of the layer into the chromatography chamber and can be collected easily. A commercial apparatus is available from Research Specialties Co. (see p. 40) for this purpose. The apparatus is similar to that shown in Figure 6.3 except that there is an opening in the bottom of the chamber.

A second approach for continuous development has been described

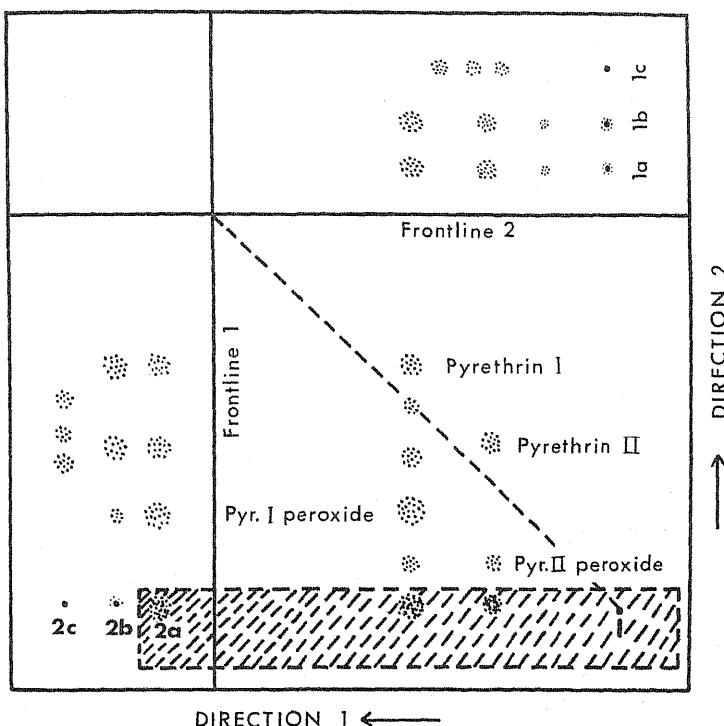


Figure 6.8. Thin-layer chromatography of pyrethrins by the separation-reaction separation-technique. The irradiation (dotted line area) was performed after the first separation. The same solvent system, 25% ethyl acetate in hexane, was used for both separations. The sample was initially placed at 1. Spots 1a, 1b and 2a and 2b are different concentrations of the starting mixture which will be developed in only one direction in order to orient the results of the two dimensional chromatogram. Spots 1c and 2c are a commercial (Desaga-Brinkmann) dye mixture of butter yellow, indophenol and Sudan Red G which is used to calibrate the layer and validate the R_f values given. The adsorbent was Silica Gel G. (Reproduced from Stahl, *Arch. Pharm.*, 293/65, 531 (1960) through the courtesy of the author and Verlag Chemie.)

by Brenner and Niederwieser.^{45,46} In this technique, the solvent is carried by a paper wick to one edge of a thin layer, passes through it and evaporates at the opposite edge. Samples cannot be taken from the end of the layer in this apparatus, which is illustrated in Figure 6.9. This has been used for the separation of amino acids⁹⁹ and for

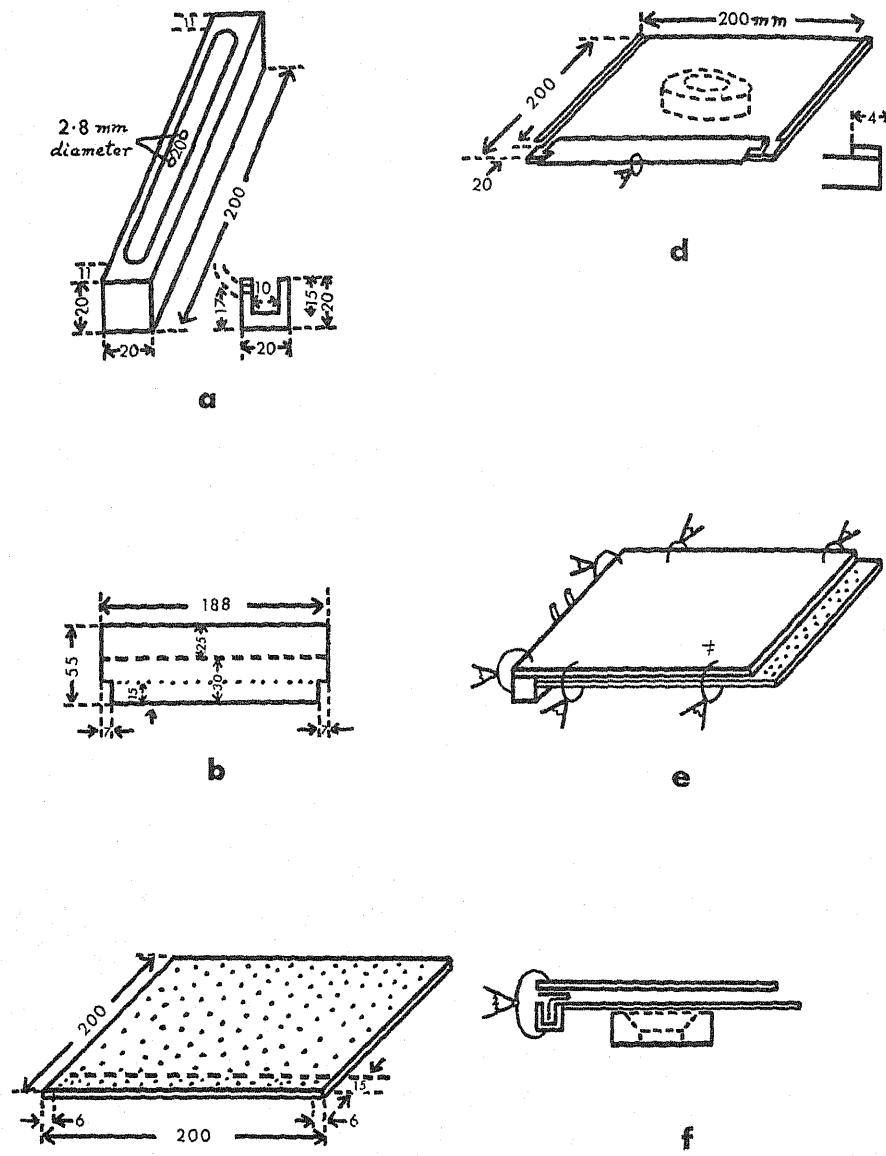


Figure 6.9. Brenner-Niederwieser apparatus for continuous, horizontal thin-layer chromatography. All measurements are in mm. Drawing a shows the steel solvent trough and the holes which feed solvent into it. Drawing b is the filter paper wick which carries the solvent to the layer. Drawing c shows a thin layer which has been spotted on a line 15 mm from the bottom and from which the adsorbent has been removed on the outermost 6 mm of the sides. Drawing d shows the layer in place on a cork ring with the wick in place and a detail of the edge strip which will support the cover plate. Drawing e shows a top view of the assembled apparatus and drawing f shows a side view (without the edge strips). In operation the sample is applied, the apparatus is assembled and the solvent is added. (Reproduced from Brenner and Niederwieser, *Experientia*, 17, 237 (1961) through the courtesy of the authors and Birkhaeuser Verlag).

preparative separations.¹⁵⁸ A commercial apparatus is available from Desaga-Brinkmann (see p. 37).

Essentially the same thing has been accomplished in a simpler fashion by developing in chambers without lids or by using a chamber shorter than the layer is long. In the latter case, a cover with a slit for the plate is used as shown in Figure 6.10a. The solvent evaporates after it emerges from the slit. The author has carried out continuous development by placing a piece of filter paper over the top of the plate as shown in Figure 6.10b. The filter paper is held in place with rubber bands or paper clips and leads the solvent over the top of the plate.

Layer Electrophoresis

Electrophoresis has been carried out on thin layers by Honegger¹⁵⁸ and by Pastuska and Trinks.²⁹¹ Honegger used Silica Gel G, Kieselguhr G and Aluminum Oxide G for the separation of amines and amino acids and combined the two processes of thin-layer chromatography and electrophoresis. That is, he separated a mixture by chromatography in one dimension and subjected the results to electrophoresis in the second dimension. Pastuska and Trinks investigated amino acids, phenols, amines and dyes. In both cases, the layer was

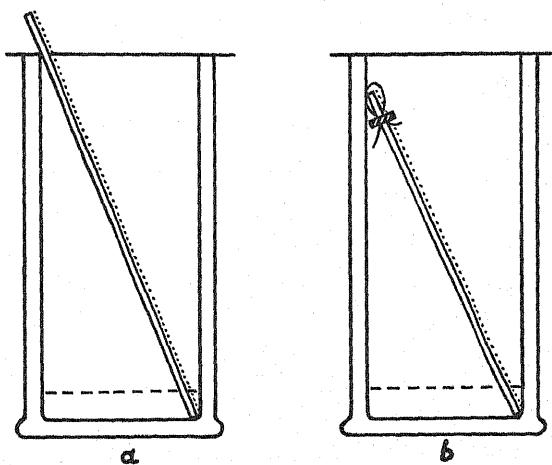


Figure 6.10. Simple arrangements for continuous thin-layer chromatography.

impregnated with an appropriate electrolyte and current was transmitted to the layer with filter paper moistened with electrolyte.

REPRODUCIBILITY OF *Rf* VALUES

One of the major disadvantages of thin-layer chromatography is that *Rf* values of substances are not exactly reproducible, at least from one laboratory to another. Furthermore, different samples of the same compound sometimes show slightly different behavior *even on the same layer*. These difficulties are more common in adsorption thin-layer chromatography and are probably traceable to the complexity of surface adsorption or to the fact that no two laboratories prepare layers in exactly the same manner. Many workers^{47,112,144,146,227,252,290} have commented extensively on this problem and their remarks will be summarized by considering the various factors which determine and affect *Rf* values.

Nature of the Adsorbent

The nature of the adsorbent and the thickness and moisture content of the layers are major factors in the determination of *Rf* values and have been discussed previously in this book. The use of standard, commercial adsorbents, layer thicknesses over 0.15 mm and consistent drying times will largely reduce the variations. It has been noted,⁴⁷ however, that there is an appreciable difference between batches of Silica Gel G. The worker should also remember that an activated layer becomes less active after exposure to a moist atmosphere for appreciable times.

Developing System

The nature of the developing system is, of course, a prime factor in any discussion of *Rf* values and should be controlled as closely as possible by the use of reagent grade solvents. Solvent mixtures should be made up frequently. This is especially true when very volatile solvents such as ether or pentane are being used. The mere opening of a chamber containing such a volatile solvent in a mixture can change the concentration appreciably.

Nature and Amount of Applied Sample

The nature of the sample, that is the properties of its components, is the third major factor in the determination of R_f values and is not controllable. The amount of sample does not have too much effect so long as it does not exceed the capacity of the system. Above this point, the R_f increases³⁵² or decreases and tailing or bearding results, depending upon whether the substance has a convex or a concave adsorption isotherm (see p. 82). The worker is urged to look at a given compound or mixture at several concentrations since the capacity of a layer will vary from one substance to another.⁴⁷

The R_f of a given substance may not be exactly the same in a mixture as it is in the pure state.²⁸⁸ If a given compound is suspected to be present in a mixture, this should be confirmed by chromatographing a pure sample against a mixture of the pure compound and the substance under investigation. The appropriate spot should increase in size if the compound is, in fact, a component.

Saturation of the Chamber

Care should be taken to saturate the chromatographic chamber with solvent as completely as possible before use.³⁶⁹ This is normally accomplished by partially lining the walls with filter paper which dips into the solvent and, when possible, by shaking the chamber before the introduction of the chromatogram.⁴⁷ Such saturation (called oversaturation by Stahl) will speed up development by about one-third,²³² will give lower, but more consistent R_f values and will produce straighter solvent fronts and rounder spots.

The so-called "edge effect,"^{42,369} that is, the difference in R_f values between the center and the edge of the plate, can largely be cured by complete chamber saturation if the layers have a fairly consistent thickness.

Multicomponent Developing Systems

There are two factors which should be watched when the developing system consists of several solvents. The first, as noted above, is the evaporation of the more volatile components and the second is the variation in concentration which will occur as the solvent front moves up the layer. To alleviate this latter situation, Brenner, Nieder-

wieser, Pataki and Fahmy⁴⁷ recommended that the ratio of the distance from the solvent level to the sample origin and the total distance traveled by the solvent front be kept constant when precise R_f values are being measured.

Temperature

Variations in temperature have little effect on thin-layer chromatography.⁴⁷ This is in contrast to the situation with paper chromatography where it is quite important. This is understandable where adsorption chromatography is concerned, but less so when the chromatography is partition.

REPORTING R_f VALUES

When reporting R_f values, it is a good idea to report the R_f values of some universally available substance or substances in the system used. The dye system of Stahl (butter yellow-indophenol-Sudan Red G)³⁶⁹ is readily available (from Desaga-Brinkmann) and is satisfactory for certain ranges of adsorption chromatography. As an alternate method, Dhont and de Rooy^{84,85} reported R_B values (sample migration/migration of butter yellow) for 2,4-dinitrophenylhydrazones and 3,5-dinitrobenzoates; Peereboom²⁹³ reports R_A values (sample migration/migration of dibutyl sebacate) for various esters used as plasticizers; and Lisboa and Diczfalussy²²¹ report R_{OE_1} values (sample migration/migration of estrone) for steroids.

TAILING

This bothersome phenomenon can normally be attributed to two factors, both of which have been discussed previously. The first, that is, the presence of various ionic species of acids and bases, can be alleviated by the addition of small quantities of acids and bases to the developing system (p. 65). The second factor is sample overloading such that one is working in the nonlinear portions of the isotherms of the substances being separated.* If the isotherms are convex, tail-

* This is discussed in considerable detail by Hagdahl on pages 63 and 64 and by Giddings and Keller on page 96 in Heftmann's book "Chromatography."¹⁴²

ing will occur in concentrations above the linear range. If they are concave, bearding or tails which precede the sample will result. In any case, the situation can be improved by working at lower concentrations.

CHROMATOGRAPHY CHAMBERS

Any wide-mouth jar or bottle which can be completely sealed will serve as a chromatography chamber. The ideal chamber is not too much larger than the plates which are to be developed so that chamber saturation is rapid and, further should have a fairly flat bottom so that the plate will be straight. Special chambers are available from all of the manufacturers of thin-layer equipment.

CHAPTER 7

Visualization

INTRODUCTION

The visualization of developed thin-layer chromatograms can be carried out in many ways. The method used in a specific case will depend upon the type of compounds to be visualized, the nature of the adsorbent and the purpose of the chromatogram. Most visualization methods involve a spray reagent, but other techniques are available.

The inorganic character of most thin layers permits the use of corrosive spray reagents such as sulfuric acid, nitric acid, chromic acid or mixtures of these. These acids will convert almost any organic compound to carbon and thereby serve as universal reagents. The applicability of such reagents constitutes one of the major advantages of thin-layer chromatography. In addition, those specific spray reagents which have been developed for paper chromatography and which do not involve a mechanical washing step can be used.

The majority of spray reagents are destructive in that they destroy or modify the materials which they visualize. In preparative thin-layer work or in quantitative work where the sample is to be eluted before assay, such destructive methods cannot be used. Techniques for surmounting this problem will be discussed in Chapters 9 and 10.

Visualization techniques involving the presence of phosphors or fluorescent substances in thin-layer adsorbents have been developed as well as methods depending upon the relative transparencies of adsorbent and adsorbed compounds when sprayed with the proper reagent. Finally, techniques of radiochemistry and bioassay have been applied to the problem of visualization.

CORROSIVE AND UNIVERSAL SPRAY REAGENTS

With the exception of iodine, these reagents are strong dehydrating and oxidizing systems which function by converting organic compounds to carbon. The reagents are sprayed on the chromatograms which are subsequently heated to about 100°, and the organic compounds appear as black spots. The system is not without flaws, however. When layers containing silver nitrate are sprayed with sulfuric acid,²⁰⁰ a very strong oxidizing system is produced which oxidizes the organic compounds to carbon dioxide. A second difficulty arises when the compound is very unreactive or volatilizes before it chars. In the case of camphor,¹⁹³ this difficulty was solved by spraying the chromatogram with concentrated sulfuric acid containing 5 per cent nitric acid. The sprayed chromatogram was placed, face down, on a glass cloth and heated on a hot plate registering approximately 500°. After the acid fumes ceased to come off, the glass plate was removed and the layer (which had come loose from the plate) was turned over with the glass cloth. The compounds showed as black spots. Such a volatility has been utilized by Baehler,¹⁰ who visualized chromatograms by subliming the resolved compounds out of the adsorbent layer on to a cooled plate held 1 mm above the heated layer (see p. 115).

Iodine, either in a spray or as a vapor, is an exceptionally fine universal reagent and has been used extensively (see Table 7.1). It appears to concentrate itself in the region containing an organic compound,^{219,244} thus producing a brown spot. The normal technique is to spray with 1% solution of iodine in methanol or to place the layer in a closed container with a few crystals of iodine. In either case, the spots fade rapidly and must be marked quickly. Under normal conditions, the reagent does not appear to modify the adsorbed substances.^{17,230,244} If one wishes more permanent spots, the layers can be sprayed with a 0.5 per cent solution of benzidine in absolute ethanol while the iodine spots are still visible.⁶

A number of universal reagents are given in Table 7.1.

SPECIFIC SPRAY REAGENTS

In general, paper chromatographic spray systems which do not require mechanical washing are applicable to thin layers. If, however,

TABLE 7.1. UNIVERSAL SPRAY REAGENTS USED IN THIN-LAYER CHROMATOGRAPHY

| Reagent | Composition and Use | Ref. |
|--|---|--|
| 1. Conc. H_2SO_4 | Spray with acid and heat to 100–110°. | 193 and many others |
| 50% H_2SO_4 | Spray with reagent and heat to 200°. Observe in daylight and U.V. | 25, 26, 27, 262, 264, 310, 420 |
| 2% H_2SO_4 in H_2O -EtOH (1:1) | Spray with reagent and heat to 100°. | 221 |
| H_2SO_4 in acetic anhydride | Spray with H_2SO_4 -acetic anhydride (1:3) and heat. | 363, 368, 378 |
| 2. H_2SO_4 -KMnO ₄ | Sol. A: 0.5 g KMnO ₄ in 15 ml conc. H_2SO_4^1 | 19, 98 |
| | a. Spray with A. | |
| | b. Heat. | |
| 3. H_2SO_4 -Na ₂ Cr ₂ O ₇ | Sol. A: 3 g Na ₂ Cr ₂ O ₇ in 20 ml H ₂ O diluted with 10 ml conc. H_2SO_4 | 98 |
| | a. Spray with A. | |
| | b. Heat. | |
| H_2SO_4 -K ₂ Cr ₂ O ₇ | Spray with sat. sol. of K ₂ Cr ₂ O ₇ in conc. H_2SO_4 and heat. | 95 |
| H_2SO_4 -chromic acid | Spray with a sat. sol. of chromic acid in conc. H_2SO_4 and heat. | 232 |
| 4. H_2SO_4 -HNO ₃ | Spray with H_2SO_4 -HNO ₃ (1:1) and heat. | 110, |
| | Spray with 5% HNO ₃ in H_2SO_4 and heat. | 190, 191, 193, 252 |
| 5. HClO ₄ | Spray with 2% (or 25%) sol. and heat to 150°. | 247, 296 |
| | Allow 70% acid to diffuse into the layer at right angle to developing direction. | 70 |
| 6. I ₂ | Spray with 1% of I ₂ in MeOH. Place in closed chamber containing I ₂ crystals. | 119, 130, 183, 186, 230, 232, 234, 235, 244, 249, 292, 361, 397, 420 |
| I ₂ -benzidine | Spray I ₂ treated layer with 0.5% sol. of benzidine in absolute EtOH. | 6 |

¹ Large quantities of KMnO₄ can lead to the formation of explosive manganese heptoxide.

Silica Gel G layers are treated with dichlorodimethylsilane,¹⁸⁵ they are stable enough to be washed. The procedure is to place the layer in a vacuum desiccator with the silane and evacuate it to 300 mm for fifteen minutes. The layer is removed and allowed to stand for

thirty minutes. It is then stable enough to be dipped in aqueous solutions.

A number of specific spray reagents are given in Table 7.2. Table 7.3 is a cross reference for Table 7.2.

Explanation of Table 7.2

Reagent. The reagents are listed alphabetically according to the major component of the reagent or, on occasion, the name of the reagent. The minor components as well as some additional names are given in the cross reference, Table 7.3.

Preparation and Use. When several similar preparations have been used, the author has chosen one to put in the table. However, the references apply to all documented applications of the reagents.

The following abbreviations are used to conserve space:

| | | | |
|-------|----------------|------------|--------------------|
| HOAc | acetic acid | PrOH | <i>n</i> -propanol |
| NaOAc | sodium acetate | BuOH | <i>n</i> -butanol |
| EtOAc | ethyl acetate | pet. ether | petroleum ether |
| EtOH | ethanol | EtOEt | ethyl ether |
| MeOH | methanol | | |

Type of Compounds. The types of compounds given cover most, but not all, of the total uses of a given reagent.

Color. The colors are generally taken from a single paper and should not be taken too seriously.

References. The references apply to the main bibliography.

METHODS INVOLVING FLUORESCENCE AND PHOSPHORESCENCE

The preparation of fluorescent and phosphorescent thin layers by the incorporation of chemicals in the adsorbent has been discussed in Chapter 2. Several fluorescent reagents such as 2',7'-dichlorofluorescein (0.2 per cent in ethanol,²³²) fluorescein (0.04 to 0.004 per cent sodium salt in water,^{144,227,366}) Rhodamine B (0.05 per cent in water,¹⁶⁵) and morin (0.05 per cent in methanol³⁴¹) can also be sprayed on thin-layer chromatograms.

In any case, the treated layers cause compounds containing conjugated double bonds to show up as colored spots when viewed in an

Thin-Layer Chromatography

TABLE 7.2. SPRAY REAGENTS

| | Reagent | Preparation and Use | Type of Compounds | Color | Ref. |
|---|---|--|---|------------------|-----------------|
| 1 | Acid Violet-7B (6BN) | Sol. A: 1.5% aqueous sol. of reagent. Do not heat over 60° a. Spray with A b. Warm to 100° for 20 min. | cations of the alkali and alkaline earth metals | varies | 315, 358 |
| 2 | Aluminum chloride | Sol. A: 5% alcoholic sol. of reagent a. Spray with A b. Observe in daylight and U.V. | flavonoids | yellow spots | 286 |
| 3 | Ammonia vapor | Expose to vapor. | flavonol glycosides | | 126 |
| 4 | Ammonium molybdate-HClO ₄ | Sol. A: 3 g of ammonium molybdate in 25 ml H ₂ O, 30 ml of 0.1 N HCl and 15 ml 60% HClO ₄ a. Spray with A b. Heat to 110° for 15 min. | universal for lipids | blue-black spots | 157 |
| 5 | Ammonium molybdate-SnCl ₂ | Sol. A: 1% aqueous sol. of ammonium molybdate Sol. B: 1% SnCl ₂ in 10% HCl a. Spray with A b. Dry c. Spray with B d. Heat to 105° | phosphates (inorganic) | blue spots | 355 |
| 5 | Aniline phthalate | Sol. A: 0.93 g aniline and 1.66 g phthalic acid in 100 ml of <i>t</i> -BuOH saturated with H ₂ O[33] a. Spray with A b. Heat to 105° for 10 min. | reducing sugars | various colors | 439 |
| 6 | Anisaldehyde in H ₂ SO ₄ and HOAc | Sol. A: 0.5 ml of reagent in 0.5 ml conc. H ₂ SO ₄ , 9 ml of 95% EtOH and a few drops of HOAc a. Spray with A b. Heat to 105° for 25 min. Sol. A: 0.5 ml of reagent in 50 ml of HOAc and 1 ml conc. H ₂ SO ₄ a. Spray with A b. Heat to 120° for 6 min. | carbohydrates steroids | various blues | 377, 441 247 |

| | | | | |
|----|---|--|--|---|
| 7 | <i>p</i> -Anisidine | Spray with an alcoholic sol. 0.1 M in both <i>p</i> -anisidine and phthalic acid | honeybee larval foods reducing sugars | 292 various colors 350 |
| 8 | <i>p</i> -Anisidine phthalate CCl_4 | Sol. A: 20% (also 12 and 16) sol. of reagent in CCl_4 a. Spray with A b. Heat to 120° c. Observe in daylight and U.V. | terpenoids, steroids and essential oils | 117, 171, 286, 299, 368, 394 |
| 9 | Antimony pentachloride in CHCl_3 | Sol. A : sat. sol. of reagent in alcohol-free CHCl_3 a. Spray with A b. Heat to 100° for 10 min. c. Observe in daylight and U.V. | steroids, steroid glycosides, aliphatic lipids, vitamin A and others | 13, 21, 67, 68, 128, 129, 141, 145, 171, 223, 247, 257, 287, 368, 371, 382, 392, 415, 416, 457, 460 |
| 10 | Antimony trichloride in CHCl_3 with acetic anhydride | Sol. A: sat. sol. of reagent in alcohol-free CHCl_3 , acetic anhydride (5:1) a. Spray with A b. Heat to 130° for 5-10 min. c. Observe in daylight and U.V. | steroids and steroid glycosides | 322 |
| | Antimony trichloride in HOAc | Sol. A: equal parts, by weight, of SbCl_3 and HOAc a. Spray with A b. Heat to 95° for 5 min. ³³ | steroids | 348 |
| | Antimony trichloride in CHCl_3 with SOCl_2 | Spray with a sat. sol. of reagent in alcohol-free $\text{CHCl}_3\text{-SOCl}_2$ (10:1) | Δ^4 -steroids | 145 |
| 11 | <i>Aqua regia</i> -ammonium molybdate-vanadyl chloride | Sol. A: <i>aqua regia</i> (3 parts conc. HCl and 1 part conc. HNO_3) Sol. B: 3 ml of 5% ammonium molybdate and 7 ml 5 N HCl diluted to 100 ml with acetone Sol. C: 2 g V_2O_5 and 20 ml conc. HCl boiled until blue green, then diluted to 37 ml with 6 N HCl and to 400 ml with H_2O ; 20 ml of this is then shaken with zinc powder until it becomes brown; decant and use immediately a. Spray with A, B and C consecutively | phosphorus in phosphates blue spots | 174 |

Thin-Layer Chromatography

TABLE 7.2.—Continued

| | Reagent | Preparation and Use | Type of Compounds | Color | Ref. |
|----|------------------------------|---|---|------------------------|-------------------------------------|
| 12 | Benzidine, diazotized | Sol. A: 0.1 to 0.085 M Na ₂ HPO ₄ Sol. B: 0.18 g benzidine in 50 ml 0.5 N HCl Sol. C: 2 ml of B diluted with 2 ml 1% NaNO ₂ , allowed to lose its color (3–5 min), treated with 2 ml of 5% urea and finally diluted with 14 ml H ₂ O a. Spray with A b. Spray with C _{14a} | phenols which couple glycolipids | violet | 288, 290, 291 127, 197, 427, 432 |
| 13 | Bial's reagent | Sol. A: 40.7 ml conc. HCl mixed with 0.1 g orcinol, 1% FeCl ₃ and diluted with H ₂ O to 50 ml a. Place in HCl atmosphere at 80° for 1.5 hr. b. Spray with A c. Place in HCl atmosphere at 80° until reaction occurs | estrogens with phenol groups | yellow | 221 |
| 14 | Boule reaction | a. Expose to NH ₃ vapor b. Expose to nitrogen dioxide (from Cu and HNO ₃) | carboxylic acids | yellow spots on green | 42, 105, 106, 107, 193 |
| 15 | Bromoresol green | Spray with a 0.3% sol. of reagent in H ₂ O-MeOH (20:80) containing 8 drops of 30% NaOH per 100 ml. | a. halogen ions, except F ⁻ b. dicarboxylic acids | yellow spots on purple | 357, 290, 291 |
| 16 | Bromoresol purple | Spray with a 0.1% sol. of reagent in EtOH made just basic with NH ₄ OH or NaOH | aliphatic carboxylic acids | yellow spots on blue | 302 |
| 17 | Bromophenol blue-citric acid | Sol. A: 0.5 g of bromophenol blue and 0.2 g of citric acid in 100 ml H ₂ O a. Spray with A | universal for lipids | yellow spots on blue | 134, 173, 174, 208, 459 |
| 18 | Bromthymol blue | I. <i>alkaline</i> Spray with a sol. of 40 mg of reagent in 0.01 N NaOH (100 ml) II. <i>buffered</i> Spray with a sol. of 50 mg of reagent and 1.25 g of boric acid in 8 ml of 1 N NaOH and 112 ml H ₂ O | | | |

| | | | | | |
|----|---|--|--|----------------------|----------|
| 19 | Ceric sulfate-trichloroacetic acid | Sol. A: 0.1 g Ce(SO ₄) ₂ suspended in H ₂ O; add 1 g of trichloroacetic acid; boil and add, drop by drop, 1.84 ml of conc. H ₂ SO ₄ until it becomes clear a. Spray with A b. Heat to 110° ^a | tocopherols | various colors | 354 |
| 20 | Chloramine T-HCl-NH ₃ | Sol. A: 10% reagent in water Sol. B: 1 N HCl a. Spray with A b. Spray with B c. Heat to drive off Cl ₂ d. Expose to NH ₃ ³⁵ | caffeine | rose red | 117 |
| 21 | Chlorine-KI-starch | a. Expose to Cl ₂ for 0.5 hr. b. Drive off excess Cl ₂ by waving in air c. Spray with sol. of KI and starch Sol. A: 160 mg toluidine in 30 ml HOAc; add 1 g KI and make up to 500 ml. a. Expose to Cl ₂ for 5-10 min. b. Allow to stand in air 3-5 min. c. Spray with A ³⁵ | esters of carbobenzoxy-amino acids | blue spots | 456 |
| | Chlorine-toluidine-KI | Sol. A: 160 mg toluidine in 30 ml HOAc; add 1 g KI and make up to 500 ml. a. Expose to Cl ₂ for 5-10 min. b. Allow to stand in air 3-5 min. c. Spray with A ³⁵ | 3-phenyl-2-thiohydantoins of amino acids | 46 | |
| 22 | Chromotropic acid in H ₂ SO ₄ | Sol. A: 10% aqueous sodium 1,8-dihydroxynaphthalene-3,6-disulfonate-23 N H ₂ SO ₄ (1:5) a. Spray with A b. Heat to 105° for 30 min. | methyleneendiox group | blue | 32 |
| 23 | Chlorosulfonic acid-HOAc | Sol. A: CISO ₃ H-HOAc (1:2) a. Spray with A b. Heat to 130° for 5 min. c. Observe in U.V. | triterpenes and cardiac aglycones | violet-brown | 405, 406 |
| 24 | Copper acetate-dithioxamide on stabilized layers | Sol. A: 20 ml of sat. aqueous copper acetate diluted to 1000 ml Sol. B: 0.1% dithioxamide in EtOH a. Dip layers in H ₂ O b. Dip layers in A for 10 min. c. Wash with H ₂ O for 30 min. d. Dip in B for 10 min. e. Wash in H ₂ O | fatty acids | green spots on white | 180 |

Thin-Layer Chromatography

TABLE 7.2—Continued

| | Reagent | Preparation and Use | Type of Compounds | Color | Ref. |
|----|---|--|--|------------------------|---|
| 25 | Cyanogen bromide-henzidine | Sol. A: 0.05% benzidine in 2 N HOAc a. Expose to CNBr vapor b. Spray with A | nicotinic acid derivatives | red to brown | 117 |
| 26 | α -Cyclodextrin-I ₂ | Sol. A: 1.0% sol. of reagent in H ₂ O-EtOH (70:30) a. Spray with A b. Expose to I ₂ | monochain lipids | white spots on purple | 230, 231, 232 |
| 27 | <i>o</i> -Dianisidine | Spray with a 1% sol. of reagent in HOAc | aldehydes and ketones | yellow-brown | 193, 252, 328, 368 |
| 28 | Dibromo-R-fluorescein | Sol. A: 0.2% sol. of reagent in EtOH a. Spray with A b. Observe in U.V. | glycerides | yellow fluorescent | 16 |
| 29 | 2',7'-Dichlorofluorescein | Sol. A: 0.2% sol. of reagent in 96% EtOH a. Spray with A b. Observe in U.V. | nonpolar lipids, saturated and unsaturated | green spots on purple | 8, 28, 120, 230, 232, 234, 235, 236, 264, 323, 334, 363, 420, 423 |
| 30 | 2,6-Dichloroquinone-chlorimide-NH ₃ | Sol. A: 0.1% sol. of reagent in EtOH a. Spray with A b. Expose to NH ₃ | Vitamin B ₆ | blue spots | 116, 117 |
| 31 | 2,6-Dichloroquinone-chlorimide-borax | Sol. A: 2% quinone in EtOH Sol. B: 2% borax in water a. Spray with A and B consecutively | phosphate esters | blue spots | 293 |
| 32 | N,N-Dimethyl- <i>p</i> -phenylenediamine-2HCl-NaOMe-irradiation | Sol. A: 0.5 g of diamine in 100 ml MeOH with 1 g Na a. Spray with A b. Moisten with H ₂ O c. Expose to U.V. without filter | chlorinated hydrocarbons | green-violet spots | 23 |
| 33 | <i>p</i> -Dimethylaminobenzaldehyde in HCl (van Urk reagent) | Sol. A: 0.1 to 1% reagent in 30 ml of conc. HCl and 50 ml 96% EtOH a. Spray heavily with reagent b. Spray lightly with <i>agua regia</i> (for alkaloids) | sulfonamides alkaloids (indole) | 452 blue rose spots | 376 |
| | <i>p</i> -Dimethylaminobenzaldehyde in H ₂ SO ₄ | Spray with 1% sol. of reagent in 2% sol. of H ₂ SO ₄ in EtOH | a. essential oils b. morphine | | 287, 437 |

| | | | | | |
|------|--|--|---|---------------------------------|--|
| 33 | 2,4-Dinitrophenylhydrazine (2,4-DNPH) | Spray with a 0.5% sol. of reagent in 2 N HCl | aldehydes and ketones | yellow to red spots | 2, 13, 105, 106, 107, 165, 171, 192, 287, 323, 326, 328, 368, 376 |
| 33a. | DNH-(reduced diphospho-pyridine nucleotide) | | enzymes | | 82 |
| 34 | Diphenylamine | Sol. A: 1% sol. of reagent in EtOH a. Spray with A. b. Expose to U.V. | nitramine explosives | blue spots | 216 |
| | Diphenylamine in HCl and HOAc | Sol. A: 10% sol. of amine in EtOH a. Dilute 20 ml of A with 100 ml conc. HCl and 80 ml HOAc b. Spray with diluted A. c. Cover with another plate and heat to 110° for 30-40 min. | glycolipids | blue | 157, 174 |
| 35 | Diphenylboric acid- β -amino-ethyl ester | Sol. A: 1% of reagent in MeOH a. Spray with A. b. Observe in U.V. | flavonols, coumarins and their glycosides | varies | 379 |
| 36 | s-Diphenylcarbazone | Spray with a 0.1% sol. of reagent in 95% EtOH | acetoxymethoxymercury adducts of unsaturated lipids | purple to blue spots on rose | 177, 232, 233 |
| 36a. | Diphenylthiocarbazone | Sol. A: 0.01% of reagent in CHCl ₃ a. Spray with A | organotin derivatives | orange-red | 411 |
| 37 | Dragendorff's reagent | I. Sol. A: 1.7 g of basic bismuth nitrate in 100 ml of H ₂ O-HOAc (80:20) Sol. B: 40 g KI in 100 ml H ₂ O a. Spray with sol. made from 5 ml of A, 5 ml of B, 20 g of acetic acid and 70 ml H ₂ O II. Sol. A: Warm 2.6 g of bismuth carbonate and 7 g of NaI in 25 ml of HOAc. After 12 hr, filter sol. and dilute 20 ml of the filtrate with 8 ml EtOAc Sol. B: 10 ml of A diluted with 25 ml HOAc and 60 ml EtOAc a. Spray with B b. Colors can be enhanced by spraying with 0.1 N H ₂ SO ₄ ³¹⁵ | alkaloids and choline containing compounds | orange | 88, 157, 188, 227, 273, 332, 344, 427, 430, 444 |

Thin-Layer Chromatography

TABLE 7.2.—Continued

| | Reagent | Preparation and Use | Type of Compounds | Color | Ref. |
|----|--|--|---|-------------------------|--|
| 38 | Ehrlich reagent | Sol. A: 1% sol. of <i>p</i> -dimethylaminobenzaldehyde in 95% EtOH a. Spray with A b. Place in HCl atmosphere for 3–5 min. | a. tryptophane metabolites b. gangliosides | various colors | 87, 208 |
| 39 | a. Echt blue salt B (tetraazotized di- <i>o</i> -enisidine) b. Echt blue salt BB (diazo-tized 1-amino-4-benzoyl-amido-2,5-dioethoxyben-zene). c. Echt red salt B (diazotized 5-nitro-2-aminoanisole) | Sol. A: 5% aqueous sol. of reagent a. Spray with A b. Spray with 0.1 N NaOH | phenols and amines, which couple | | 288 |
| 40 | Ferric chloride Ferric chloride in HOAc | Spray with a 1% aqueous sol. of reagent Sol. A: Equal volumes of 5% aqueous FeCl ₃ and 2 N HOAc a. Spray with A | phenols pyrazolones | various colors | 287, 292 |
| 40 | Ferric chloride-K ₄ Fe(CN) ₆ | Sol. A: 1 volume 5% aqueous K ₄ Fe(CN) ₆ , 2 volumes 10% aqueous FeCl ₃ and 3 volumes H ₂ O a. Spray with A | phenacetin phenols chlorinated hydrocarbons | blue 117 98 43 | 227 |
| 41 | Ferrous ammonium sulfate-potassium thiocyanate | Spray with a mixture of the two aqueous solu-tions | peroxides | | 242 |
| 42 | Fluorescein | Sol. A: 0.04% aqueous sol. of sodium fluorescein a. Spray with A b. Observe in U.V. | conjugated double-bond systems | | 82, 227 |
| | Fluorescein-Br ₂ | Sol. A: 0.04% aqueous sol. of sodium fluorescein a. Spray with A b. Observe in U.V. for conjugated systems c. Expose to Br ₂ d. Observe in U.V. for unsaturates | unsaturated compounds | yellow spots on pink | 105, 106, 107, 171, 190, 191, 192, 193, 232, 252, 328, 365, 368 |

| | | | | |
|----|--------------------------------------|---|----------------------------|----------------|
| 43 | Folin-Ciocalteau reagent | Sol. A : Mix 10 g sodium tungstate, 2.5 g of sodium molybdate and 70 ml H_2O . Add, consecutively, 5 ml 85% H_3PO_4 and 10 ml conc. HCl . Reflux for 10 hr and add 15 g Li_2SO_4 , 5 ml H_2O and 1 dp Br_2 . Dilute to 100 ml with H_2O . Reagent should not be green. Sol. B: 20% aqueous Na_2CO_3 a. Spray with B b. Dilute A with 3 volumes H_2O and spray ³⁷⁵ | a. phenols b. estrogens | 163, 221, 380 |
| 44 | Fuchsin- H_2SO_4 | Sol. A: 1 g parafuchsin is dissolved in 700 ml H_2O and 50 ml 2 N HCl, diluted to 1 l with H_2O and allowed to stand overnight with occasional shaking. a. Add 1% $HgCl_2$ to A b. Spray with reagent ⁴⁸ | plasmalegens | 134 |
| 45 | Hydrazine sulfate in HCl | Spray with a 1% sol. of reagent in 1 N HCl | aromatic aldehydes | Yellow |
| 46 | Hydrogen chloride | Hold over conc. HCl | chalcones | red |
| 47 | Hydroxamic acid-ferric ion | Sol. A: equal volumes of 12.5% NaOH and 5% hydroxylamine, both in H_2O Sol. B: acetic acid Sol. C: 10% $FeCl_3$ in H_2O a. Spray with A b. Allow to stand for 10 min (or heat to 100° for 10 min) c. Spray with B and C consecutively | esters of all types | varies |
| 48 | 8-Hydroxyquinalidine-NH ₃ | Sol. A: 0.5% sol. of reagent in 60% EtOH a. Expose to NH_3 b. Spray with A c. Observe in U.V. | inorganic cations | various colors |
| | | | | 359, 360 |

Thin-Layer Chromatography

TABLE 7.2—Continued

| | Reagent | Preparation and Use | Type of Compounds | Color | Ref. |
|----|--|---|---------------------------|----------------|------|
| 49 | Iodine-potassium iodoplatinate | Sol. A: 0.5% sol. of I ₂ in CHCl ₃ Sol. B: equal volumes of 1.1% aqueous KI and 0.135% aqueous chloroplatinic acid a. Spray with A and B alternating | nitrogen-containing drugs | yellow | 279 |
| | Iodine in KI and HOAc | Sol. A: aqueous sol. which contains 5% I ₂ and 10% KI a. Dilute 2 ml of A with 3 ml H ₂ O and 5 ml 2 N HOAc and spray with diluted sol. | alkaloids and steroids | 347, 227 | |
| | Iodine-benzidine | Sol. A: 5% benzidine in EtOH a. Expose to I ₂ vapor b. Spray with A | glycerides | blue | 6 |
| 50 | Iodine azide | Sol. A: 3.5 g of sodium azide in 100 ml of 0.1 N I ₂ (reagent is explosive when dry ⁴⁵) a. Spray with A | penicillins | yellow | 103 |
| 51 | Isatin in H ₂ SO ₄ | Sol. A: 0.4% sol. of reagent in conc. H ₂ SO ₄ a. Spray with A b. Heat to 120° | thiophene derivatives | various colors | 65 |
| 52 | isonicotinic acid hydrazide | Sol. A: 4 g isonicotinic acid hydrazide and 5 ml conc. HCl in 1 l H ₂ O a. Spray with A b. Allow to stand 3-16 hr. c. Observe in U.V. and daylight | steroids | | 414 |
| 53 | Kedde reagent | Sol. A: 2% sol. of 3,5-dinitrobenzoic acid in MeOH Sol. B: 2 N KOH a. Mix equal volumes of A and B just before spraying b. Spray with mixture | cardiac glycosides | | 223 |

| | | | | | |
|----|--|--|---|---------------------------------------|--|
| 54 | Morin (2',3,4',5,7-pentahydroxyflavone) | Sol. A: 0.05 to 0.005% sol. of reagent in MeOH a. Spray with A. b. Heat to 100° for 2 min. (optional) c. Observe in U.V. | a. N-blocked amino acids b. conjugated double-bond systems | yellow | 52, 341 |
| 55 | N-(1-Naphthyl)ethylene diamine·2 HCl (diazonized) | Sol. A: 1 N HCl Sol. B: 5% NaNO ₂ Sol. C: 0.1% reagent in EtOH a. Spray with A, B, and C (consecutively) | sulfonamides | reddish purple | 196 |
| 56 | Naphthoresorcinol in H ₃ PO ₄ | Sol. A: 100 ml of 0.2% sol. of reagent in EtOH and 10 ml H ₃ PO ₄ a. Spray with A. b. Heat to 105° for 5-10 min. | carbohydrates | various colors depending upon type | 120, 289, 307, 308 |
| 57 | Ninhydrin | I. Sol. A: 95 ml of 0.2% reagent in BuOH plus 5 ml of 10% aqueous HOAc a. Spray with A. b. Heat to 120-150° for 10-15 min. II. Sol. A: 50 ml of 0.2% sol. of reagent in abs. EtOH mixed with 10 ml of HOAc and 2 ml of 2,4,6-collidine Sol. B: 0.1% Cu(NO ₃) ₂ ·3H ₂ O in abs. EtOH a. Spray with A-B (50:3) mixed just before use b. Heat to 105° | a. amino acids b. aminophosphonates c. amino sugars | blue | 44, 95, 99, 116, 117, 134, 156, 292, 424, 438, 450 |
| 58 | Osmium tetroxide | Expose to reagent vapors (caution, poison) in closed chamber | cmpds. with double bonds | brown to black | 459 |
| 59 | Palladium chloride in HCl | Spray with a 0.5% sol. of reagent in dilute HCl | thiophosphate insecticides | yellow spots on brown | 23 |
| 60 | Periodic acid-HClO ₄ -V ₂ O ₅ | Sol. A: 10 g H ₄ IO ₆ in 100 ml of 70% HClO ₄ and a few crystals of V ₂ O ₅ a. Spray carefully dried layer with A Sol. A: 0.5% HIO ₄ in 90% HOAc Sol. B: 30% sol. of sodium pyrosulfite-3 N HCl (1:1) (both kept at 0°) Sol. C: 5 ml of 10% sodium pyrosulfite, 85 ml H ₂ O and 0.2 g tuchsin. Allow sol. to stand 12 hr, treat with carbon and filter a. Spray with A, B, and C consecutively b. Heat to 90° for 15 min. | thiophosphate esters glycerides | blue | 304 |
| | Periodic acid-Schiff's reagent | | | violet | 174 |

Thin-Layer Chromatography

TABLE 7.2.—Continued

| | Reagent | Preparation and Use | Type of Compounds | Color | Ref. |
|----|--|--|--|------------|--|
| 61 | Phosphomolybdic acid | Sol. A: 1.5% (also 5 and 10%) sol. of reagent in EtOH or EtOH-EtOEt (1:1) a. Spray with A b. Heat to 150° for 5 min. | a. bile acids b. glycerides c. unsaturated fatty acids d. many others | blue | 13, 115, 152, 154, 155, 157, 179, 181, 182, 183, 184, 186, 238, 249, 293, 295, 362 |
| | Phosphomolybdic acid-SnCl ₂ | Sol. A: 1% sol. of acid in CHCl ₃ -EtOH (1:1) Sol. B: 1% SnCl ₂ in 2 N HCl a. Spray with A b. Dry and dip in B | phospholipids | blue | 185 |
| 62 | Phosphomolybdic acid-NH ₃ | Sol. A: 20% sol. of acid in EtOH a. Spray with A b. Dry for 2-3 min and expose to NH ₃ | tocopherols | blue | 354 |
| 63 | Photophotungstic acid | Sol. A: 10% sol. of acid in EtOH a. Spray with A b. Heat to 100° for 5-10 min. c. Observe in U.V. | cholesterol esters | | 249 |
| 64 | Phosphoric acid | a. Spray with 50-70% acid b. Heat to 100° for 10-30 min. c. Observe in U.V. | pregnanetriols and other steroids | | 247, 391, 414 |
| 65 | <i>ortho</i> -Phosphoric acid-phosphomolybdic acid | Sol. A: 40% <i>ortho</i> -phosphoric acid Sol. B: 5% sol. of phosphomolybdic acid a. Spray with A b. Heat to 110° for 7 min. c. Observe in U.V. d. Spray with B e. Heat to 110° for 2 min. | steroids | blue-green | 433, 434, 435 |

| | | | | |
|----|---|---|---|--|
| 66 | Potassium hydroxide | Sol. A: 5% KOH-acetone (2:1) a. Spray with A b. Heat to 100° c. Cool and spray (optional) with $\text{HNO}_3\text{-H}_2\text{SO}_4$ (1:1) | aromatic nitro compounds and amines | 110 |
| | Potassium hydroxide-urea | Sol. A: 1 N KOH in EtOH Sol. B: 50% aqueous sol. of urea a. Spray with A b. Heat to 60° for 15 min. (citrates are yellow) c. Spray with B d. Observe in U.V. | citrates | 293 |
| 67 | Potassium iodide | Spray with saturated aqueous sol. Sol. A: 2% sol. of KI in H ₂ O a. Spray with A b. Observe colors c. Expose to NH ₃ d. Expose to H ₂ S | peroxides copper series of inorganic cations | 242 359 |
| | Potassium iodide-HOAc-Na ₂ SO ₃ -starch | Sol. A: fresh sol. of 5 ml of 4% KI, 20 ml HOAc and a few drops of 1% Na ₂ SO ₃ Sol. B: 1% aqueous sol. of starch a. Spray with A and B consecutively | peroxides | 371 |
| 68 | Potassium iodoplatinate | Sol. A: mix 45 ml of 10% KI with 5 ml of 5% platinum chloride and dilute to 100 ml a. Spray with A | alkaloids and vitamins | various colors 57, 116, 117, 279, 285, 426 |
| 69 | Potassium permanganate (alkaline) | Spray with 0.25% aqueous sol. of reagent Sol. A: equal portions of 1% aqueous KMnO ₄ and 2% aqueous NaClO ₃ a. Spray with A b. Note color after 1-2 hr. | diterpenoids phenols and plasticizers (esters) | 213 290, 293 |
| | Potassium permanganate-HOAc | Spray with mixture of equal volumes of 0.1 N KMnO ₄ and 2 N HOAc | oxidizable materials | 227 |
| | Potassium permanganate-Fe(No ₃) ₃ | Sol. A: 0.5% aqueous sol. of KMnO ₄ Sol. B: 1% aqueous sol. of Fe(No ₃) ₃ a. Spray with A and B consecutively | phenols | 340 |

TABLE 7.2—Continued

| | Reagent | Preparation and Use | Type of Compounds | Color | Ref. |
|----|---|---|-------------------------|----------------------|--|
| 70 | Procházka reagent | Sol. A: 10 ml of 35% formaldehyde, 10 ml of 25% HCl and 20 ml 96% EtOH a. Spray with A b. Heat to 100° c. Observe in daylight and U.V. | indole derivatives | yellow | 376 |
| 71 | Pyridyl-azo-naphthalol | Spray with a 0.25% sol. of reagent in EtOH | uranium ion | | 360 |
| 72 | Resorcinol-SnCl ₂ -H ₂ SO ₄ -KOH | Sol. A: 20% resorcinol in EtOH with added SnCl ₂ Sol. B: 4 N H ₂ SO ₄ Sol. C: 40% aqueous KOH a. Spray with A b. Heat to 150° for 10 min. c. Spray with B d. Heat to 120° for 20 min. e. Spray with C | esters and plasticizers | various colors | 293 |
| 73 | Rhodamine B | Spray with a 0.05% (or 0.5%) sol. of reagent in EtOH | lipids | purple spots on rose | 19, 165, 181, 182, 183, 232, 427, 430, 448 |
| | Rhodamine B-KOH | Sol. A: 0.05% aqueous sol. of reagent Sol. B: 10 N KOH a. Spray with A and B consecutively | glycerides | bright spots on rose | 6 |
| 74 | Rhodamine 6 G | Sol. A: 0.05% aqueous sol. of reagent a. Spray with A b. Observe in U.V. | lipids | | 459 |
| 75 | Silver nitrate-NH ₂ NaOMe | Sol. A: 0.3% AgNO ₃ in MeOH Sol. B: MeOH saturated with NH ₃ Sol. C: 7% Na in MeOH a. Mix 5 parts of A, 1 part of B and 2 parts of C b. Spray with mixture c. Note color after few min. ^{61a} | acyl sugar | brown | 75 |
| 76 | Silver nitrate-NH ₄ OH-fluorescein | Sol. A: 1% sol. of AgNO ₃ made basic with NH ₄ OH Sol. B: 0.1% sol. fluorescein in EtOH a. Spray with A and B consecutively | halogen ions | | 357 |

| | | Spray with a 1% aqueous sol. of reagent | ferrocenes | blue | 345 |
|----|--|--|--|---------------|-----------------------------|
| 77 | Sodium periodate | Sol. A: 4 parts of 2% aqueous NaIO_4 and 1 part of 1% KMnO_4 in 2% aqueous Na_2CO_3 a. Spray with A ^{51a} | sugars | | 454 |
| 78 | Sodium periodate-K MnO_4 | Sodium periodate-ethylene glycol-thiobarbituric acid Sol. A: 0.02 M NaIO_4 , Sol. B: ethylene glycol-acetone-H 2SO_4 (50:50:0.3) Sol. C: 6% aqueous sol. of 2-thiobarbituric acid sodium salt a. Spray with A, B, and C consecutively. ^{45b} | neuraminic acid sialic acid derivatives | red | 127 |
| 79 | Stannous chloride-HCl | Spray with a 10% sol. of SnCl_2 in conc. HCl | aromatic nitro compounds | blue | 110 |
| 80 | Sudan Black B on stabilized layers | Sol. A: 0.1% sol. of reagent in EtOH-H 2O (1:1) a. Dip in A b. Wash with EtOH-H 2O (1:1). | saturated glycerides | blue | 180 |
| 81 | Sulfanilic acid, diazotized (Fauly's reagent) | Sol. A: 0.5 g of sulfanilic acid and 0.5 g of KNO_2 in 100 ml of 1 N HCl I. a. Spray with A b. Spray with 1 N NaOH II. a. Spray with conc. KOH in MeOH b. Spray with A | phenols and aromatic amines which will couple | yellow-orange | 11, 72, 74, 82, 396, 426 |
| 82 | Tetrazolium blue or 3,3'-(3,3'-dimethoxy-4,4'-biphenylene)-bis[2,5-di-phenyl-2H-tetrazolium chloride] ¹ | I. Reducing steroids Sol. A: 1% sol. in EtOH Sol. B: Mix just before using 2 ml of A, 5 ml of H 2O and 3 ml of 4 N NaOH a. Spray with A II. Δ^4 -3-oxosteroids Sol. A: 90 ml 2.7 N NaOH and 0.1 ml of sol. A above. a. Spray with A ^{51a} | corticoids | | 1, 243 |
| 83 | 2,3,5-triphenyltetrazolium chloride-NaOH | Sol. A: Mix just before use one volume of 4% sol. of reagent in MeOH and one volume of 1 N NaOH a. Spray with A b. Heat to 110° for 5-10 min. ³⁵ | a. steroids b. reducing sugars c. Glycosides | red spots | 30, 247, 414 |

TABLE 7.2—Continued

| | Reagent | Preparation and Use | Type of Compounds | Color | Ref. |
|----|---|---|--|---|-------------------|
| 84 | Trichloroacetic acid | Sol. A: 4% sol. of reagent in CHCl ₃ a. Spray with A b. Allow to stand | menthofuran K-strophanthin (in U.V.) | pink reddish-brown | 19 223 |
| | Trichloroacetic acid-chloramine-T | Sol. A: Mix just before use 3 volumes of a 25% sol. of acid in EtOH with 2 volumes of 3% chloramine-T sol. a. Spray with A b. Heat to 100° for 10 min. c. Observe in daylight and U.V. | cardenolides | | 90, 223, 322 |
| 85 | Trinitrobenzene | | azulenes | | 108 |
| 86 | Uranium acetate | Spray with a 1% sol. of reagent in H ₂ O | flavonoids | brown | 286 |
| 87 | Vanillin-H ₂ SO ₄ | Sol. A: 1% sol. of reagent in conc. H ₂ SO ₄ a. Spray with A. b. Observe in daylight and U.V. | terpenoids | various colors | 49, 167, 168, 368 |
| | Vanillin-H ₃ PO ₄ | Sol. A: 1% sol. of reagent in 50% H ₃ PO ₄ a. Spray with A. b. Heat to 120° for 10-20 min. | steroids | | 247, 336, 338 |
| 88 | Water on Silica Gel G | a. Spray thoroughly b. Observe in reflected light | alkaloids, steroids, sugar acetates and others | opaque areas on semi-transparent background | 115, 128, 398 |
| 89 | Zwicker's reagent | Sol. A: 0.5% sol. of cobalt acetate in MeOH Sol. B: 0.5% sol. of lithium hydroxide in MeOH a. Spray with A and B consecutively | barbiturates | | 227 |

TABLE 7.3. CROSS REFERENCES TO SPECIFIC SPRAY REAGENTS

| Reagent or Name | Item in 7.2 | Reagent or Name | Item in 7.2 |
|---|-------------|-------------------------|-------------|
| Acetic anhydride | 10 | Iodine | 26 |
| Benzidine | 25 | Lithium hydroxide | 89 |
| Bismuth nitrate | 37 | Pauly's reagent | 81 |
| Bismuth carbonate | 37 | Perchloric acid | 4 |
| Borax | 30 | Potassium ferricyanide | 40 |
| Bromine | 42 | Potassium iodide | 21 |
| Chloramine-T | 84 | Potassium iodoplatinate | 49 |
| Cobalt acetate | 89 | Potassium permanganate | 78 |
| 2,4,6-Collidine | 57 | Potassium thiocyanate | 41 |
| <i>p</i> -Dimethylaminobenzal-dehyde | 38 | Pyruvic acid | 33a |
| 3,5-Dinitrobenzoic acid | 53 | Schiff's reagent | 60 |
| Diphospho-pyridine nucleotide (reduced) | 33a | Sodium molybdate | 43 |
| Dithiooxamide | 24 | Sodium tungstate | 43 |
| Dithizone | 36a | Stannous chloride | 4, 72 |
| Ferric chloride | 47 | Starch | 21 |
| Fluorescein | 76 | Thiobarbituric acid | 78 |
| Formaldehyde | 70 | Thionyl chloride | 10 |
| Hydrogen sulfide | 67 | Toluidine | 21 |
| Hydroxylamine-HCl | 47 | Trichloroacetic acid | 19 |
| | | Urea | 66 |
| | | van Urk's reagent | 32 |

ultraviolet light.²³² If fluorescein sprayed layers are then treated with bromine vapor, the fluorescein is converted to eosin except where unsaturated compounds were present. The result is that the unsaturated compound shows up as a yellow spot on a pink background. It is best viewed in ultraviolet light.^{227,232,366}

TRANSPARENCY METHODS

If a developed layer is sprayed with or dipped into a liquid of the correct refractive index, the adsorbent and the compounds to be visualized will have relatively different transparencies and can be distinguished when held in a correct angle with respect to light. In this manner, spots or bands can be located without destruction and with great accuracy. The most commonly used system of this type is water on silica gel,^{115,128,398} in which the bands or spots stand out as semiopaque areas on a translucent background. The method would obviously fail if the adsorbed materials were water soluble.

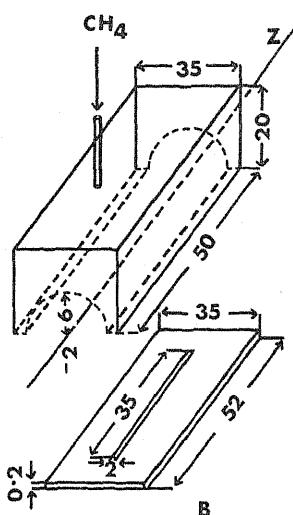


Figure 7.1. Gas flow counter with an extremely flat aperture plate (B) for measurement of weak β -emitters on thin-layer chromatograms. The aperture plate is attached to the counter tube without projecting parts. Z = counter wire (30μ diameter). The measurements are given in mm. (Reproduced from Schulze and Wenzel, *Angew. Chem. Intern. Ed. Engl.*, 1, 580 (1962) through the courtesy of the authors and the publishers, Verlag Chemie and Academic Press.)

In a variation of this technique, Kaufmann, Makus and Khoe¹⁸⁵ dipped Silica Gel G layers (which had been stabilized with dichlorodimethylsilane) in a mixture of ethanol-water (2:8) so that triglyceride spots became transparent. Hefendehl¹⁴¹ sprayed already visualized Silica Gel G layers with a paraffin-ether mixture (1:1). The paraffin-impregnated layers became transparent after being stored in a desiccator for a few hours and the colored spots were easier to photograph.

RADIOACTIVITY METHODS

Chromatograms of radioactive compounds can be visualized by making X-ray prints.^{20,232,234a} The layers are directly exposed to "No-Screen Medical X-Ray Safety Film"** for times varying from a few hours to a week. The films are developed with "Supermix Developer"*** for 4 to 6 min. and fixed for 30 min. with "Acid Fixer." These methods have been applied mostly to lipids, although radioactive halides have also been detected by radioactivity.²⁹

Schulze and Wenzel³⁴⁰ developed a method for continuous auto-

* Eastman Kodak Company, Rochester 3, N.Y.

** General Electric X-Ray Dept., Milwaukee 1, Wisc.

matic measurement of radioactivity distribution of weak β -emitters on thin layers. Accurate measurements of C¹⁴ and tritium-containing compounds were made by passing the developed layer slowly under the aperture of the especially designed gas-flow counter shown in Figure 7.1.* The gas was methane. The method was also used for locating radioactive materials in preparative thin layer chromatography.

BIOASSAY METHODS

The developed chromatogram is covered with an agar plate containing an appropriate microorganism in good health. The spots which contain antibiotic inhibit the microorganisms and can thereby be detected. Thus, Nicolaus, Coronelli and Binaghi^{275,276} located a number of penicillins, tetracyclines and rifomycins using *Sarcina lutea* and *Bacillus subtilis* in the presence of triphenyltetrazolium salts. The sensitivity was 0.01 to 0.1 γ . The macrolides have been determined³³ using *Bacillus subtilis* as the revealing organism.

* A commercial model is available from Laboratorium Prof. Dr. Berthold, Wildbad (Schwarzwald) Germany.

CHAPTER 8

Documentation of Results

INTRODUCTION

Thin-layer chromatograms are normally too fragile for preservation as such and several techniques have been devised for making a permanent record of them. In general, these are to trace them or draw them in a laboratory book, to photograph or photocopy them, or to imbed them in a paraffin layer or plastic film of some type. The latter two, more exact, documentation methods offer the additional advantage that the photograph or films can often be quantitized.

PHOTOGRAPHY

The most positive way to record thin-layer chromatograms is to photograph them.^{46,137,141,223,262,273,328,352,416} Chromatograms which are visible in normal light can be photographed with any available camera system. Hefendehl¹⁴¹ found that better pictures resulted when the chromatograms were made semitransparent after normal visualization procedures (see p. 104).

Thin-layer chromatograms can also be copied with an office letter-copying device such as the "Zerox 914,"*¹⁴⁸ "Photorapid"**¹²¹ or the "Copease" apparatus.†²³² A process using a direct positive blueprinting paper, Driprint HC (F speed)‡ has been suggested.⁹⁶ In some

* Manufactured by Zerox Corp., Rochester 3, N.Y.

** Manufactured by Bürogeräte, A. G., Zürich, Switzerland, Pronto model with Gevalcopy paper GS, 8½ × 11 in.

† Manufactured by Copease Corp., 425 Park Avenue, New York, N.Y.

‡ E. Dietzgen Co., 407 10th Street, N.W., Washington, D.C.

cases^{96,121} the layer is covered with cellophane film before photography to protect both the layer and the photocopying apparatus. It has also been suggested¹⁴⁸ that better copies will result if the spots are outlined with a stylus or even completely removed before duplication.

Sylvania F8T5/BLB, "Blacklite" blue tubes* and a yellow filter are recommended by Bürke and Bolliger²³² for black and white photography in ultraviolet light. Lukas has made photographs in ultraviolet light using an ultraviolet filter,²²³ and Hansbury, Langham and Ott¹³⁷ have devised a method using a "Polaroid" system for the same purpose.

The use of X-ray film for recording chromatograms of radioactive substances was discussed in Chapter 7 (p. 104).

METHODS OF LAYER PRESERVATION

Meinhard and Hall²⁴⁶ pressed a piece of "Scotch" or cellophane tape on thin layers to remove a portion of the layer. The tape was then placed directly in a laboratory book. The applicability of this technique is governed by the visualization procedure and the spray reagents used.

Mottier^{265,267} and Machata²²⁷ prepared semipermanent records of chromatograms by imbedding the layer in paraffin. In the case of unbound adsorbents, paraffin is applied to the plate as though it were another developing solvent, that is, by dipping the lower part of the layer in the melted paraffin and allowing it to rise by capillary action.^{265,267} The bound layers are impregnated by dipping them in melted paraffin.²²⁷ Semipermanent records can also be made by covering the layer with another glass plate and thus preserving it as a sandwich between two plates.^{227,232} The sandwiches are sealed by taping them around the edges.

A more permanent preparation can be made by imbedding the adsorbent layer in a plastic film which can be peeled off of the glass plates and preserved as such. This is accomplished by spraying the chromatogram with a 4 per cent collodion solution containing 7.5

* Sylvania Electric Products, Inc., 500 5th Ave., New York, N.Y.

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per cent glycerol,¹⁸ aqueous dispersions containing 10 to 15 per cent of polyacrylic acid esters, polyvinylidene chloride and polyvinyl propionate,²¹⁸ Fisher Label Glaze,*²³² or "Neatan" (Brinkmann, see p. 37).

* Fisher Scientific Company, 633 Greenwich Street, New York, N.Y.

CHAPTER 9

Preparative Thin-Layer Chromatography

INTRODUCTION

The preparative separation of reasonable amounts of a mixture (10 to 1000 mg) on thin layers has been a relatively neglected aspect of thin-layer chromatography. The high capacities of the adsorbents (when used in adsorption chromatography), the high degrees of resolution obtained, and the over-all simplicity of the method make it a near ideal tool for the organic chemist. The technique itself involves the mechanical steps of normal thin-layer chromatography (Chapters 2 to 8) with slight modifications.

Thus, the layers are thicker (0.5 to 2 mm compared with 0.25 mm) than normal so that the maximum amount of material can be separated at once. The sample is not applied in spots but in a narrow band and developed in such a manner that the resolved components lie in bands. These bands are located by some nondestructive visualization technique; the adsorbent containing a single component is removed from the band area and the sample is removed from the adsorbent by elution with an appropriate solvent. Quantities ranging from 10 to 250 mg can be separated on a single chromatogram by this technique and a number of chromatograms can conveniently be carried out simultaneously.

The development of this aspect of thin-layer chromatography has been severely hampered by the lack of methods for sample application and for the nondestructive visualization of the developed chro-

matogram. The sample must be applied in large quantities to a relatively thin band without undue destruction of the rather fragile layer. It now appears that satisfactory, though not ideal, solutions have been found for these problems.

At least two attempts have been made to apply the concepts of thin-layer chromatography to large-scale separations. These involve chromatography in cast blocks or "chromatobars"²⁵⁰ of plaster-of-Paris-bound adsorbent and chromatography in horizontal cellophane tubes filled with adsorbents.⁸⁶ A third similar technique is chromatography on layers of cellulose one inch thick packed between glass plates.¹³⁵ While each of these methods has certainly been successful in itself, the author believes that they are generally more laborious and inferior to the technique outlined below.

The various aspects of preparative thin-layer chromatography will be dealt with in the order and in the same manner as has been done in the preceding chapters for the general technique. Numerous examples of preparative work are cited as such in the tables of Chapter 11.

ADSORBENTS

Both bound and unbound adsorbents can be used with no special treatment other than perhaps a prepurification step (see pp. 19 and 48).

PREPARATION OF LAYERS

For the sake of convenience, most preparative thin-layer chromatography has been carried out on the usual square two-dimensional plates (20 × 20 cm or 8 × 8 in.). Larger plates could, of course, be used to separate larger quantities, but their use becomes awkward above this size and commercial apparatus is not available for preparation of the layers.

The adsorbent slurries have usually been made in the normal manner although Honegger suggests¹⁵⁸ the use of somewhat less water and a little more binder for very thick layers (see Table 3.2). Ritter and Meyer³²⁹ found the optimum layer thickness to be about 1 mm

although Honegger¹⁵⁸ gives a somewhat wider range of 1 to 3 mm. The very thick layers tend to crack and there is an appreciable difference between behavior on the open side of the layer and the side next to the glass. Layers up to 2 mm thick can be made with the adjustable Stahl applicator (see p. 37) and layers up to 5 mm thick can be made with the Camag apparatus (see p. 36).¹⁵⁸

The layers are best allowed to dry at room temperature for several hours before final activation. This prevents casehardening and subsequent cracking. It is further recommended that, as the original Desaga instructions suggest, the layers be dried in a vertical position rather than horizontal.

SAMPLE APPLICATION

Sample application is made by applying the sample, in an appropriate solvent, in a thin band about 2 cm from one edge of the layer. A smooth application can be effected using the mechanical devices of Morgan²⁵⁸ or of Ritter and Meyer³²⁹ as discussed in Chapter 4. Alternately, the sample can be applied from a syringe moved manually along the layer. The operation is best carried out on a heated metal block^{223,329} to evaporate the solvent. Honegger¹⁵⁸ applied the sample in a V-shaped trough cut in the layer.

The amount of sample applied will depend upon the specific mixture to be separated, the adsorbent involved and upon the type of chromatography. Much larger samples can be separated by adsorption than by partition chromatography. Mangold states²³² that up to 50 mg can be separated by adsorption chromatography on a layer of Morgan²⁵⁸ or of Ritter and Meyer³²⁹ as discussed in Chapter 4. Meyer³²⁹ separated 75 mg on a plate of the same size with a 1-mm-thick layer. Honegger states¹⁵⁸ that 5 to 25 mg can be separated on such a 1-mm layer and Černý, Joska and Lábler⁵² separated 20 to 100 mg of steroids on a layer of unbound alumina 20 × 10 cm and 1 mm thick. The author considers it "safe" to apply 50 mg of any mixture to a layer 20 × 20 cm and 1 mm thick and has applied as much as 300 mg of an easily separated mixture. For partition chromatography, only about one-tenth of these sample sizes should be used. Mangold²³² suggests 5 mg on a 20 × 20 cm layer 0.25 mm thick.

CHOICE OF DEVELOPMENT SOLVENTS

The solvents for preparative chromatography are the same as those used for normal thin layers.

DEVELOPMENT

The development of preparative chromatograms is carried out in the normal way with the following precautions. If several chromatograms are developed simultaneously, special care must be taken to saturate the chamber. The author prefers to develop such chromatograms with adsorbent turned toward the chamber wall and close to, but not touching, a layer of filter paper on the wall. If several chromatograms are to be developed in sequence in the same chamber, it should be remembered that thick layers remove more solvent than thin layers and the composition of the solvent may change appreciably.

All of the tricks of development discussed in Chapter 6 except those involving two-dimensional chromatography are applicable. Multiple-step and continuous development are particularly useful.¹⁵⁸

VISUALIZATION

In the ideal case, the bands on a developed chromatogram will be colored or can be seen in ultraviolet light.^{52,224} Bands containing conjugated double-bond systems can be located by using an adsorbent containing phosphors or a fluorescent additive as discussed in Chapter 2 (p. 21). Sometimes, small amounts of fluorescent or colored impurities will be present in a sample which will allow the orientation of a preparative plate with respect to a small plate (preferably with the same layer thickness) which has been visualized in a normal destructive manner. Schulze and Wenzel³⁴⁹ used their gas-flow counter to find bands of compounds containing C¹⁴ and tritium labels.

Mistryukov²⁵⁵ describes the use of "paper prints" to locate zones. Narrow paper strips (5 to 6 mm) are moistened and pressed against an unbound layer. After removal, the strips contain a layer of adsorbent and can be visualized with any spray reagent.

Iodine appears to be a good visualization reagent for most lipids²³⁰

since it does not affect them appreciably. Gritter and Albers¹²⁸ have located steroids and other types of compounds by spraying the layer with water as discussed in Chapter 7 (p. 103). When all of these methods fail, an edge spray technique can be used. The preparative layer is covered with a glass plate except for 2 to 3 cm along one vertical edge and a line of adsorbent is scraped off along the edge of the cover plate to isolate the narrow edge strip.³²⁹ The edge is then sprayed with an appropriate reagent, and the results are extrapolated across the layer.^{230,333,420, and others}

ELUTION OF COMPOUNDS

Most workers simply scrape the adsorbent containing the desired components from the glass plate with a spatula and elute it with an appropriate solvent. However, this is difficult when using unbound layers and two gadgets have been devised for the purpose. Both operate on the vacuum cleaner principle. The first, as shown in Figure 9.1, is a piece of glass tubing with a constriction and holds a cotton wad to catch the adsorbent. It was devised by Mottier^{267,269} and has the advantage that it can be used as an elution tube and no sample is lost in transfer. The Ritter and Meyer apparatus,³²⁹ as shown in Figure 9.2, is more suitable for large quantities. The adsorbent is sucked into a Soxhlet extractor thimble from which it is eluted by extraction.

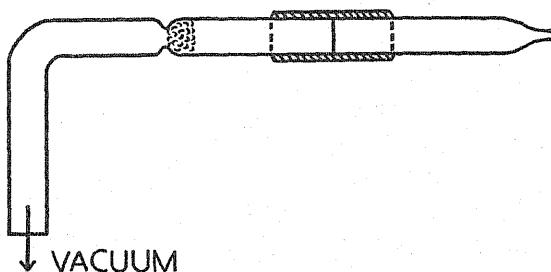


Figure 9.1. Mottier gadget for removing adsorbent from a thin layer. (Reproduced from Mottier, *Mitt. Gebiete Lebensm. Hyg.*, 49, 454 (1958) through the courtesy of the author and the Eidgenoessische Drucksachen und Materialzentrale, Bern, Switzerland.)

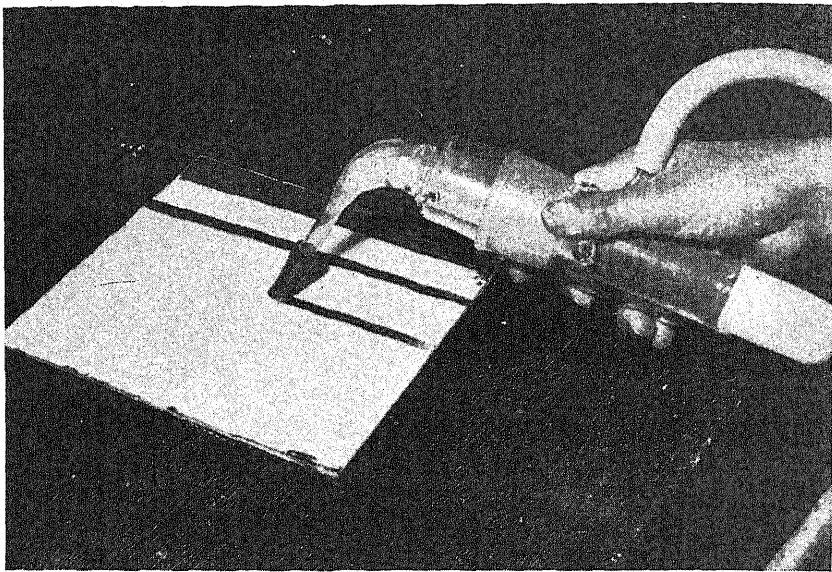


Figure 9.2. Ritter and Meyer apparatus for removing adsorbent from a thin layer.
(Reproduced from Ritter and Meyer, *Nature*, 193, 941 (1962) through the courtesy of the authors and Macmillan and Co., Ltd.)

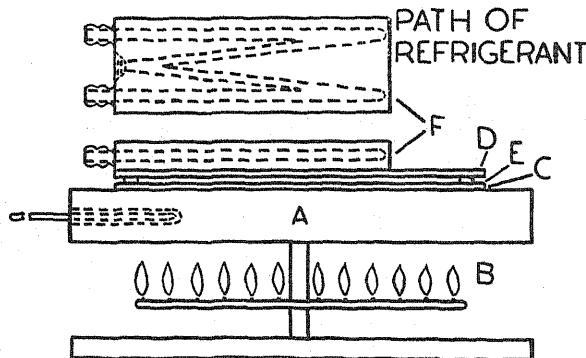


Figure 9.3. The apparatus of Baehler for direct sublimation out of thin layers. A refrigerant is passed through the metallic block (F) which rests on cover plate (D). The 1 mm spacer separates the cover plate from plate (C) which bears the thin layer. Block A is a piece of metal containing a thermometer which is heated by burners (B). (Reproduced from Bachler, *Helv. Chim. Acta*, 45, 309 (1962) through the courtesy of the author and Verlag Helvetica Chimica Acta, Basel, Switzerland.)

The ideal elution solvent is one which moves the sample with the solvent front on a thin layer.²³² Aqueous eluents cannot be used with plaster-of-Paris-bound adsorbents because they will dissolve appreciable amounts of calcium sulfate. It is essentially impossible to avoid the solution of small amounts of silica gel in any eluting solvent. If the eluent is evaporated to dryness and taken up in a fresh solvent, preferably less polar than the original, most of the adsorbent will remain on the sides of the flask.

Sometimes, the substances on a chromatogram can be removed by direct sublimation to a cooled plate held about 1 mm over the adsorbent layer. Bachler¹⁰ used the apparatus shown in Figure 9.3 for the visualization of chromatograms by sublimation, but the principle is mentioned for the purpose of isolation by Ener (as reported by Mangold²³²).

CHAPTER 10

Quantitative Thin-Layer Chromatography

INTRODUCTION

Quantitative evaluation of thin-layer chromatograms can be divided into two general categories. In the first, the mixture components are assayed *on* the thin layer while in the second, they are eluted from the adsorbent before being measured. Either technique can be used with about 5 per cent error or, in ideal cases, about 3 per cent error.

The assay on the layer can be carried out by measuring the spot area, or the spot area and density on a developed and visualized chromatogram. These values are then related to the amount of substance in the spots through the use of standards or calibration curves. Spot size or density can also be measured on photocopies or photographs of visualized chromatograms and related to substance amounts. Finally, the assay can be carried out visually¹⁹⁴ by measuring the minimum visable amount of the standard on a chromatogram and making successive dilutions of an unknown solution until this minimum value is reached. The major advantages of these techniques are their simplicity and the fact that the chromatograms can be visualized with destructive spray reagents.

The elution of substances from the adsorbent introduces several sources of error which will be discussed. A spectrophotometric or colorimetric method must be available for the assay of the eluted compounds since the simple weighing of eluted samples gives poor re-

sults.⁴⁴² The elution technique has been used more extensively than the layer assay method.

ANALYSIS ON THIN LAYERS

Spot Area

This type of determination is based upon the fact that there is a mathematical relationship between the spot area for a given substance and the weight of the substance contained in the spot. The method involves only a small number of mechanical operations and is widely applicable.^{234,290,314,315,326,353,354}

There has, however, been some controversy over the type of mathematical relationship existing between these quantities. In fact, Stahl³⁷⁴ questioned the existence of any precise relation at all. Seher^{353,354} ignored the mathematics and obtained results with only 5 per cent error using calibration curves obtained by plotting spot area (from photostats) against weight. The calibration curves were not straight lines. Brenner and Niederwieser⁴⁴ and Pastuska and Petrowitz²⁹⁰ obtained a linear relationship by plotting the logarithm of the weight of sample against spot area. This agrees with Giddings and Keller,¹²³ who derived such a relationship for paper chromatography from theoretical considerations. The most thorough and successful study of this technique was made by Purdy and Truter,^{314,315} who found a linear relationship between the logarithm of the weight of a substance and the square root of the spot area. These authors noted, however, that a reasonably linear relationship resulted when the spot area or the logarithm of the spot area was used rather than its square root.

Purdy and Truter explored three general methods of analysis based upon spot size. Using quantities ranging from 1 to 100 γ , they carried out analyses for potassium and magnesium ions, hexadecanol, cholesterol, palmitic acid, cholestryl laurate and phenylazo-2-naphthol. Both adsorption and partition chromatography were involved. In all cases, it was necessary to have a pure sample of the substance in question for use as a standard.

The Graphical Method of Purdy and Truter. This method is simi-

lar to that of the Seher^{353,354} technique, that is, it is based upon the preparation of a calibration curve by plotting spot area against weight with no regard to linearity. A standard deviation of 4.3 per cent (25 observations) was obtained and the major error was ascribed to the difficulty of duplicating conditions on the calibration chromatograms and the analysis chromatograms. In contrast, the second and third methods are algebraic and depend upon the linear relationship between $\log W$ and \sqrt{A} .

First Algebraic Method of Purdy and Truter. In this method, a standard solution of the compound to be assayed is spotted on the layer with a solution of the unknown and a solution prepared by precise dilution of the unknown. The chromatogram is developed and visualized and the spot areas are determined. The results are calculated using Equation (1) where W and A are the weight and spot area of the unknown, W_s and A_s are the weight and area of the standard, A_d is the area of the diluted unknown spot and d is the dilution fac-

$$\log W = \log W_s + \left(\frac{\sqrt{A} - \sqrt{A_s}}{\sqrt{A_d} - \sqrt{A}} \right) \log d \quad (1)$$

tor. In essence, the unknown and its diluted sample establish the slope of the straight line used for the analysis and the standard establishes its intercepts. This method is the most accurate of the three, and yielded standard deviations of 2.7 per cent (540 observations) for adsorption chromatography and 3.6 per cent (982 observations) for partition chromatography.

Second Algebraic Method of Purdy and Truter. This method requires a pure sample of the substance to be assayed rather than a standard solution. The unknown solution is diluted in a precise manner and the diluted solution is divided into two portions. A known amount of the pure substance is added to one of the diluted samples and all three solutions are spotted on the same chromatogram. The chromatogram is developed and visualized; the spot areas are determined and the results are calculated using Equation (2). In this case,

$$\log \left(\frac{Wd + a}{W} \right) = \left(\frac{\sqrt{A_+} - \sqrt{A}}{\sqrt{A_d} - \sqrt{A}} \right) \log d \quad (2)$$

W and A are the weight and spot area of the unknown; A_d and A_+

are the spot areas of the diluted sample and the diluted sample containing added substance, respectively; a is the amount of pure substance added to the diluted unknown and d is the dilution factor. This method is somewhat less accurate and yielded standard deviations of 3.9 per cent (374 observations) for adsorption chromatography and 3.9 per cent (982 observations) for partition chromatography.

Purdy and Truter found that it was necessary to spot the same volume of each solution on a single chromatogram. They used an Agla micrometer syringe suspended 2 mm above the thin layer for this purpose. The appropriate sample was squeezed out of the syringe and transferred to the layer by raising the layer until it contacted the solvent drop on the syringe. Chloroform was found to be an unsatisfactory spotting solvent. The chromatograms were developed in the usual manner and those containing colorless compounds were visualized by spraying with conc. sulfuric acid and charring at 160° for 10 minutes. The areas were determined by laying a sheet of transparent paper on the chromatograms, tracing the outline of the spots and measuring the areas of the figures by superimposing the tracing on millimeter graph paper.

In a related method, Eble and Brooker⁹³ measured the length of the streaks (actually the distance between the origin and the zone front) formed when tryptamine was chromatographed on Kieselguhr G with acetone-water (99:1). The method gave results that were reproducible to about 5 per cent.

Spot Area and Density

The size and intensity of spots formed by the acid spray and charring of a chromatogram provide a satisfactory basis for quantitative evaluation.^{296,310,311} The method has an advantage in that the densities of the spots can be measured on a densitometer.

Additional variants are added, however, with respect to the spot-area methods discussed above. These are the carbon density of a given compound, its volatility and whether it chars in a uniform manner. These disadvantages can largely be nullified by the judicious use of internal standards and calibration curves⁸¹⁰ and results can be obtained with about 5 per cent error.

Before spotting, the thin layer, either the standard size of 5×20 cm^{310,311} or microscope slide size,²⁹⁶ is scanned in the densitometer to determine the degree and the uniformity of the background density. The chromatograms are then spotted, using a micropipette or micro-syringe, with the sample containing the substance to be assayed. The chromatogram is developed, sprayed with 50 per cent sulfuric acid or 25 per cent perchloric acid and charred in an oven. It is then scanned by passing it through a densitometer and the amount of unknown substance is calculated using an appropriate calibration curve. The densitometers which have been used are the Photovolt* models 52C and 521A with a stage attached for semiautomatic plotting of curves for the standard size layers^{310,311} and the Photovolt model 501A with transmission density unit no. 52 for the microchromatoplates.²⁹⁶ The slit sizes were 1×5 mm for standard size and 1×3 mm for microsize. Glycerides^{310,311} and cholesterol²⁹⁶ have been analyzed by this technique.

Spot Area and Density on Photostats or Photographs

The spot area has been determined from photostats of thin-layer chromatograms and used as a quantitative measurement by Kammerer (as reported in Mangold²³²) and by Seher.^{353,354} The error was about 5 per cent.

Neubauer and Mothes²⁷³ photographed chromatograms of opium alkaloid mixtures and cut the photographs into strips which were passed through a densitometer. Hefendehl¹⁴¹ made chromatograms transparent (see p. 104), photographed them and measured the density of the photographs against a calibration curve. He obtained results with about 5 per cent error in the analysis of menthofuran in an essential oil. Rybicka³³⁵ followed the glycerolysis of linseed oil by this method and was able to determine the optimum reaction conditions by finding the concentrations of the glycerides at timed intervals.

Minimum Spot Visibility

In this technique,¹⁹⁴ a standard solution of the substance to be assayed is successively diluted and each dilution is chromatographed un-

* Photovolt Corp., 95 Madison Avenue, New York, N.Y. A commercial densitometer, especially designed for thin-layer work, is now available from Photovolt.

til the minimum visible amount is determined. This will, of course, depend upon the visualization method and the eye of the analyst and will vary from one compound to another. The unknown solution is then successively diluted and chromatographed until the minimum visible amount is determined. Knowing the dilution factor of the unknown and the minimum value, the amount of unknown in the original sample can be calculated. The average error for nineteen samples was 11.3 per cent.

Using a similar method, Waldi has carried out analyses for pregnandiol in urine as an early test for pregnancy^{433,435} and for adrenaline (as its triacetyl derivative⁴³⁴) in adrenal extracts. He chromatographed the solution containing the material to be assayed on the same layer with a series of standard known concentrations and estimated the concentration of the unknown by comparison.

ANALYSIS AFTER ELUTION

Analysis by Spectrophotometry

Those compounds which possess a visible or ultraviolet spectrum are conveniently assayed in the following manner. After development of the chromatogram, the spot of adsorbent containing the substance to be analyzed is quantitatively removed; the substance is eluted with an appropriate solvent and assayed in a spectrophotometer. Thus, the spectroscopic properties of such compounds provide both a means of locating the spots on a developed chromatogram and a means of quantification. Substances which contain ultraviolet chromophores are more easily observed on phosphor-containing adsorbents (p. 21).

The methods involving elution contain several more sources of error than those in which compounds are assayed on the thin layers. First, the spots on the chromatogram must be located. This, however, is a minor problem when the substances have visible or ultraviolet chromophores. Second, the adsorbent containing the substance to be assayed must be completely removed from the glass support plate. Finally, the substance must be completely removed from the adsorbent before it is measured.

It has been suggested^{194,383,389} that the thin layers be prewashed

before being used for this type of analysis. This is carried out by allowing solvent to ascend (or descend) through the layers, perhaps in an apparatus similar to that shown in Chapter 3 (p. 49). The chromatogram is then spotted with a precise amount of substance to be separated and developed in a normal manner. The spots are located by viewing them in an appropriate light and the adsorbent containing the compound to be assayed is removed, normally with a razor blade. The compound is eluted from the adsorbent with a sufficiently polar solvent (one which will move the sample to the solvent front of a chromatogram) and measured in a spectrophotometer. The gadget described by Mottier²⁶⁹ (Figure 9.1) could well be used in this technique since it provides both a method for the removal of adsorbent from the glass plate and an elution tube, thus minimizing transfer losses. The method requires the preparation of a calibration curve and compounds which follow Beer's law. It is also necessary to make "blank" readings on the adsorbent, preferably on adsorbent which has been developed in a thin layer and is removed from a point on the layer comparable to the one containing the sample to be assayed.

Normally, the method gives results with an error of 3 to 5 per cent. It was first used for the assay of biphenyl in citrus fruits (2.8 per cent error^{194,380}) and has since been used for analysis of reserpine (about 5 per cent error³⁴⁴), coumestrol (5 per cent error²²²), menthyl salicylate,³⁸³ estrogens (standard deviation, 10 per cent³⁹⁴), alkaloids (5 per cent error⁴⁰⁰), bile acids (standard deviation, 3 per cent¹¹⁵), and *p*-hydroxybenzoic acid esters (3 per cent error¹¹⁸). Quinine alkaloids²⁷⁰ and 7-geranoxycoumarin⁴¹⁷ have been eluted and determined by fluorescence methods.

Analysis by Colorimetry

This technique is almost identical with the spectroscopic method described above except that a color-producing reagent is added to the sample before it is measured in the spectrophotometer. It is subject to the same disadvantages, although the location of the substances on the developed chromatogram is now a major problem. In general, the same nondestructive visualization techniques that are discussed in

Chapter 9 (p. 112) for preparative chromatography are applicable for the location of the spots to be eluted.

The method generally gives results with about 5 per cent error. It has been used for the analysis of esters (about 5 per cent error⁴²⁰), pregnanetriols (about 10 per cent error³⁹¹), citral (2 per cent error³⁸⁶), coumarin compounds³⁸⁸ and plasma phospholipids (about 5 per cent error¹³⁴). Further, the method has been successfully applied to the assay of carbohydrates,^{120,289} steroids,^{244,278,301,460} lipids^{413,430,458} and meprobamate.¹⁰²

RADIOACTIVITY METHODS

Analysis techniques which depend upon radioactivity require prior labeling of the compounds to be analyzed. The chromatograms are spotted and developed in the usual way and have been evaluated by three methods. The first method is to make autoradiographs^{231,234a} as described in Chapter 8 (p. 104) and to evaluate them using a densitometer and calibration curve. The second method^{234a} is to locate the spots by such an autoradiograph or by nondestructive spray reagents and to elute the sample and measure it in a conventional counter. Scintillator solutions have been used^{35,349,364} to aid the counting. Finally, Schulze and Wenzel³⁴⁰ used a gas-flow counter (Figure 7.1) for the assay of C¹⁴ and tritium labeled compounds with about 3 per cent error.

The application of labeling techniques to lipids has been thoroughly discussed by Mangold.^{232,234a}

CHAPTER 11

Specific Applications of Thin-Layer Chromatography

INTRODUCTION

This chapter will contain, in a tabular form, the majority of the specific applications of thin-layer chromatography which were recorded prior to January 1, 1963. Specific *Rf* values will not be given because of their notorious inconstancy on thin layers and because they are too numerous. It is hoped that this chapter will serve at least two purposes.

First, it will provide a general idea of the types of compounds which have been chromatographed on thin layers and, to some extent, the conditions of the separation. It would be hazardous, however, to assume that this compilation is complete because chromatographic conditions are usually incidental to a piece of research and are frequently buried in the experimental description.

Second, it will provide some idea of the adsorbents, solvents and visualization techniques which are commonly used in a given area or with a given class of compounds. In many cases, the same group of compounds has been treated in more than one fashion and the reader can choose the one which best suits his facilities and inclinations.

The numerous blank spaces in the tables are due to the failure of the original author to give specific details or to the present author's inability to obtain or translate the original article. In either case, they are given to make the compilation as complete as possible.

In many cases, a single reference will give details of several separate experiments such as the use of two adsorbents, the use of two techniques or the separation of two distinct groups of compounds. In other cases, two papers will report different aspects of the same experiments. This has been shown, as well as possible, by a system of partial lines. On occasion, a dotted line is used. This means that the regions on the ends of the dotted line are related and divided as shown but that the category crossed by the dotted line is the same for both experiments.

(EXPLANATION OF TABLES II.1 TO II.23)

Compounds

The various applications have, first of all, been arranged according to the class of compounds involved. This has been somewhat arbitrary at times and considerable overlapping could not be avoided. This is particularly serious between the sections devoted to amines, alkaloids and drugs and pharmaceuticals and between the essential oils and the terpenoids. In most cases, the applications are cross-referenced and the same example is reported in two places when it involves compounds falling in two classes. When the number of compounds in a specific entry is small, they are given by name. Otherwise, collective terms are used.

Within a given class, an attempt has been made to group the applications as much as possible according to the compounds involved. In most cases, the large comprehensive papers are given first and the others follow. The papers which came to the author's attention after the tables were first prepared, however, are given at the end of each section. The 2,4-DNPH's referred to in several cases mean the 2,4-dinitrophenylhydrazones.

Adsorbent

The adsorbent is given as a specific industrial product when it is reported as such in the original paper and when it is *especially prepared for thin-layer chromatography*. When a specific product is not named, it is implied that the original authors prepared their own ad-

sorbent and the reader is referred to Chapter 2. In most cases where partition chromatography is involved, the adsorbent is equilibrated with the developing solution or some of its components. This is generally noted.

The various materials which have been incorporated into the adsorbent for one reason or another are given in the tables, but without details. The reader is referred to Chapter 2.

Developer

The solvents used for development are given as such except when the article gives a very large number. In these cases, a selected few are given, and the number not listed is mentioned in the last item. Unless noted, the different solutions represent separate experiments and are not successive.

The proportions of the various solvent mixtures, unless noted, are volume-volume. The abbreviations listed on p. 87 have been used to conserve space. Otherwise, the names or, sometimes, the formulas (CCl_4 , CHCl_3 , H_2O , etc.) are used.

Visualization

For the sake of brevity, only the names of the spray or visualization reagents are given. The reader is referred to the alphabetical listing in Chapter 7 for exact details. Two abbreviations are used in this section; 2,4-DNPH for 2,4-dinitrophenylhydrazine and U.V. for ultraviolet visualization.

Comments

The author has tried to give the basic purpose of the chromatography in each case and has, on occasion, added notes about especially interesting applications.

TABLES 11.1-11.23 FOLLOW

TABLE II.I. ALDEHYDES, KETONES AND THEIR DERIVATIVES

| | Compound | Absorbent | Developer | Visualization | Comments | Ref. |
|---|---|--------------------------------------|--|---|--|------|
| 1 | Unsaturated aliphatic aldehydes and acyl- oins | Silica Gel G | a. pet. ether-EtOEt (90:10) (95:5) b. pet. ether-EtOEt-HOAc (70:30:1) | I ₂ vapor | a. diagnostic b. monitored a syn- thesis | 119 |
| 2 | Long-chain (over eight carbons) aliphatic ketones | Silica Gel G | a. benzene-EtOEt mixtures b. toluene-EtOEt mixtures c. pet. ether-EtOEt mixtures | phosphomolybdic acid | diagnostic | 238 |
| 3 | Biologically active aldehydes | | | | | 447 |
| 4 | β -Dicarbonyl com- pounds | starch-bound silica gel | benzene-EtOAc (7:3) | | diagnostic | 14 |
| 5 | Cyclohexanodiones | see "Miscellaneous" No. 12 | | | | 254 |
| 6 | Aliphatic and aromatic aldehydes and ketones | unbound aluminum os- ide | a. benzene b. benzene-EtOH (98:2)(95:5) (90:10) c. CHCl ₃ d. EtOEt e. pet. ether-benzene (1:1) | reagents used for paper chromatography | diagnostic | 144 |
| 7 | Aromatic aldehydes and ketones | plaster-of-Paris-bound silica gel | hexane-EtOAc (4:1)(3:2) | | diagnostic | 113 |
| 8 | Phenolic aldehydes | see "Phenols, etc." No. 12 | | | | 290 |
| 9 | Vanillin and other substituted benzal- dehydes | Silica Gel G | a. pet. ether-EtOAc (2:1) b. hexane-EtOAc (5:2) c. CHCl ₃ -EtOAc (98:2) d. decalin-CH ₂ Cl ₂ -MeOH (5:4:1) | a. hydrazine sulfate in HCl b. methanolic KOH c. methanolic KOH fol- lowed by diazotized sulfanilic acid | diagnostic | 396 |

| | | | | | | |
|----|--|--|--------------------------------|--|-----|--|
| 10 | Cholestane-dione-1,3 | see "Steroids" No. 45 | | | | |
| 11 | Aldehydes and ketones in peppermint oil | see "Essential Oils" No. 6 | | | | |
| 12 | Acetals and their determination in thin layers | | | | 206 | |
| 13 | 2,4-Dinitrophenyl-hydrazines of aldehydes | Silica Gel G a. benzene-pet. ether (3:1) for aliphatic b. benzene-EtOAc (95:5) for aromatic | colored | | 84 | |
| 14 | 2,4-DNPH's of ketones | starch-bound SiO ₂ -Bentonite (4:1) a. benzene b. benzene-pet. ether-EtOEt mixtures | colored | | 209 | |
| 15 | 2,4-DNPH's of aliphatic aldehydes and ketones | Silica Gel G benzene-EtOAc (95:5) | a. colored b. alcoholic KOH | | 258 | |
| 16 | 2,4-DNPH's of aliphatic aldehydes | polyvinyl alcohol-bound silica gel benzene-H ₂ O (91:8:8:2) | colored | | 284 | |
| 17 | 2,4-DNPH's of aldehydes and ketones | unbound aluminum oxide a. benzene-hexane (1:1) b. EtOEt | colored | | 331 | |
| 18 | 2,4-DNPH's of aliphatic aldehydes | starch-bound ZnCO ₃ small amounts of pyridine in pet. ether-benzene mixtures | colored | | 9 | |
| 19 | 2,4-DNPH's of hydroxy aldehydes and ketones | a. Aluminum Oxide G b. Silica Gel G toluene-EtOAc (3:1)(1:1) | colored | | 5 | |

Thin-Layer Chromatography

TABLE 11.2. ALKALOIDS

| | Compound | Adsorbent | Developer | Visualization | Comments | Ref. |
|---|--|--|---|--|--|------|
| 1 | Fifty-four misc. alkaloids | Silica Gel G | a. $\text{CHCl}_3\text{-acetone-diethylamine}$ (3:4:1) b. $\text{CHCl}_3\text{-diethylamine}$ (9:1) c. cyclohexane- $\text{CHCl}_3\text{-diethylamine}$ (5:4:1) d. cyclohexane-diethylamine (9:1) e. benzene-EtOAc-diethylamine (7:2:1) | potassium iodoplatinate | a. diagnostic b. devised an identification system | 436 |
| | | Aluminum Oxide G | f. CHCl_3 g. cyclohexane- CHCl_3 (3:7) plus 0.05% diethylamine | | | |
| | | Silica Gel G-NaOH | h. MeOH | paper chromatography reagents | diagnostic | 144 |
| 2 | Thirty-seven misc. alkaloids | unbound aluminum oxide | a. benzene-EtOH mixtures b. CHCl_3 c. EtOH | paper chromatography reagents | diagnostic | |
| 3 | Twenty-one indole derivatives | Silica Gel G | a. MeOAc-2-propanol-NH ₃ OH (Ca. 25% NH ₃) (45:35:20) b. $\text{CHCl}_3\text{-HOAc}$ (95:5) | a. Van Urk reagent b. Prochasko reagent c. 2,4-dinitrophenylhydrazine | a. diagnostic b. two dimension | 376 |
| 4 | <i>Amaryllidaceae</i> , <i>Papaveraceae</i> and Bisbenzylisoquinoline alkaloids (total 57) | Silica Gel G Silica Gel G-NaOH | EtOAc- $\text{CHCl}_3\text{-MeOH}$ (20:20:10) | Dragendorff's reagent | diagnostic | 83 |
| 5 | Alkaloid-containing drug mixtures | Silica Gel G with fluorescent material | a. MeOH-HOAc-EtOEt-benzene (1:18:60; 120) b. EtOH-HOAc-benzene (12:5:80) c. EtOEt- $\text{CHCl}_3\text{-MeOH}$ (50:50:1) d. benzene-EtOH (9:1) e. BuOH-HOAc (10:1) | a. $\text{FeCl}_3\text{-K}_3\text{Fe}(\text{CN})_6$ b. chloramine T-HCl-NH ₃ c. potassium iodoplatinate d. 2,6-dichloroquinone chlorimide e. ninhydrin f. SbCl_5 in CCl_4 g. CNBr and benzidine | diagnostic | 117 |

| | | | | | | |
|----|---|--|---|--|--|------------|
| 6 | Alkaloids and barbiturates in toxicology | Silica Gel G | a. MeOH b. CHCl ₃ -Et ₂ OEt (85:15) | a. Dragendorff's reagent b. I ₂ and KI in HOAc c. sodium fluorescein d. FeCl ₃ in HOAc e. Zwikker's reagent f. KMnO ₄ in HOAc | a. diagnostic b. clinical | 226 227 |
| 7 | Sixteen alkaloids and nitrogen containing drugs | Silica Gel G | a. EtOH-pyridine-dioxane-H ₂ O (50:20:25:5) b. EtOH-HOAc-H ₂ O (60:30:10) c. EtOH-dioxane-benzene-NH ₄ OH (5:40:50:5) d. MeOH-BuOH-benzene-H ₂ O (60:15:10:15) Aluminum Oxide G | potassium iodoplatinate | a. diagnostic b. urine assay | 57 |
| 8 | Opium alkaloids | alumina buffered to pH 5 with acetate | H ₂ O-EtOH-BuOH (1:1:9) | U.V. | diagnostic | 239 |
| 9 | Opium alkaloids | Silica Gel G | benzene-MeOH (8:2) | Dragendorff's reagent | a. diagnostic b. quantitative c. preparative | 273 |
| 10 | Ergot alkaloids | a. cellulose powder impregnated with formamide b. Silica Gel G | a. benzene-heptane-CHCl ₃ (6:5:3) | a. Dragendorff's reagent b. With formamide layers the excess formamide was first destroyed by spraying with 0.25% NaNO ₂ in 0.5% HCl and Dragendorff's reagent was used. c. U.V. d. K ₃ Fe(CN) ₆ | a. diagnostic b. quantitative | 399 400 |
| | Morphine alkaloids | c. Silica Gel G-NaOH d. Cellulose powder impregnated with formamide | b. CHCl ₃ -EtOH (9:1) c. benzene-heptane-CHCl ₃ -diethylamine (6:5:3:0.03) | | | |
| 11 | Alkaloids of <i>Athaea</i> species | Silica Gel G | CHCl ₃ -MeOH (1:2) | a. Potassium iodoplatinate b. H ₂ SO ₄ | diagnostic | 285 |

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TABLE 11.2—Cont.

| | Compound | Absorbent | Developer | Visualization | Comments | Ref. |
|----|--|------------------------------------|---|---|--|------|
| 12 | Lysergic acid alkaloids | | | | diagnostic | 151 |
| 13 | <i>Aspidosperma</i> alkaloids | Silica Gel G (not activated) | Methyl cellosolve | U.V. | diagnostic | 215 |
| 14 | Quinine alkaloids | Silica Gel G (prepared in acetone) | "Shellisol" (23:9:9) | T-diethylamine-acetone exposed to formic acid vapor and U.V. | a. diagnostic b. quantitative | 270 |
| 15 | An isomer of rhoecadin from <i>Papaver rheas</i> | Aluminum Oxide G | a. benzene-CHCl ₃ (1:2) b. benzene-tetrahydrofuran (95:5) c. CHCl ₃ -MeOH (9:1) | Dragendorff's reagent | diagnostic | 444 |
| 16 | Morphine | Silica Gel G | BuOH-HOAc (9:1) saturated with H ₂ O | <i>p</i> -dimethylaminobenzaldehyde in H ₂ SO ₄ | a. diagnostic b. on microscopic slides | 437 |
| 17 | Tropine and <i>pseudo</i> tropine | Silica Gel G | BuOH-NH ₄ OH (28% NH ₃) (8:2) | Dragendorff's reagent | diagnostic | 188 |
| 18 | Anafrine (a piperidine alkaloid) | Silica Gel G | a. MeOH-CHCl ₃ -diethylamine (1:9:0.5) b. EtOH-NH ₄ OH (conc.) (8:2) | Dragendorff's reagent | diagnostic | 332 |
| 19 | Reserpine | Silica Gel G | MeOH-methyl ethyl ketone-heptane (8:4:33, 6:58) | U.V. | a. diagnostic b. quantitative c. developed in dark | 344 |

TABLE 11.3. AMINES AND THEIR DERIVATIVES

| | Compound | Adsorbent | Developer | Visualization | Comments | Ref. |
|---|---|-----------------------------------|---|--|--|------------|
| 1 | Simple aliphatic amines | Silica Gel G | a. EtOH (95%)·NH ₄ OH (25% NH ₃) b. phenol-H ₂ O (8:3) c. butanol-HOAc-H ₂ O (4:1:5) | ninhydrin | diagnostic | 399 |
| | | Silica Gel G buffered to pH 6.8 | d. EtOH-H ₂ O (70:30) | | | |
| 2 | Strong-base amines | unbound aluminum oxide | a. acetone-heptane (1:1) b. CHCl ₃ /NH ₃ (sat. at 22°)-EtOH (96%) (30:1) c. acetone-MeOH-H ₂ O (8:2:1) d. methyl ethyl ketone-H ₂ O (15:1) | I ₂ vapor-U.V. | a. diagnostic b. describes descending development with unbound layers | 235 256 |
| 3 | Diphenylamine derivatives | Silica Gel G | a. CHCl ₃ b. toluene c. benzene | NaNO ₂ and H ₂ SO ₄ | a. diagnostic b. analysis of powder | 138 |
| 4 | Tryptamine | Kieselguhr G | acetone-H ₂ O (99:1) | FeCl ₃ -K ₄ Fe(CN) ₆ | quantitative | 93 |
| 5 | Aromatic amines and nitro compounds | Plaster-of-Paris-bound silica gel | | a. KOH in acetone b. NaCl in conc. HCl c. HNO ₃ -H ₂ SO ₄ | diagnostic | 110 |
| 6 | Nitramine explosives | see "Miscellaneous", No. 10 | | | | 216 |
| 7 | Piperidoles and quinolols | see "Miscellaneous", No. 12 | | | | 254 |
| 8 | Twelve pteridines | Silica Gel G | a. dimethylformamide-H ₂ O (190:10) (20:1:80) b. 5% citric acid c. NH ₄ OH (25% NH ₃) d. twenty others | U.V. | diagnostic | 274 |
| 9 | Simple amines and diamines by electrophoresis | Silica Gel G buffered with borax | electrolyte: EtOH-H ₂ O (30 ml. plus 30 ml.) with 2 g. NaOAc | ninhydrin | diagnostic | 291 |

TABLE 11.4. CARBOHYDRATES, GLYCOSIDES AND THEIR DERIVATIVES

| | Compounds | Adsorbent | Developer | Visualization | Comments | *Ref. |
|---|-----------------------|---|---|--|--|-------|
| 1 | Thirteen misc. sugars | Kieselguhr G buffered with NaOAc | EtOAc-2-propanol-H ₂ O (65:23:4:11:6) | anisaldehyde and H ₂ SO ₄ | diagnostic | 377 |
| 2 | Simple sugars | Polyacrylomethyl-Kieselguhr G-Aluminum Oxide G (5:6:1) buffered with NaHPO ₄ | n-amyl alcohol-2-propanol-HOAc-H ₂ O (55:15:110:30) | aniline phthalate | diagnostic | 36 |
| 3 | Simple sugars | Silica Gel G buffered with boric acid | a. benzene-HOAc-MeOH (1:1:3) b. methyl ethyl ketone-HOAc-MeOH (3:1:1) | naphthoresorcinol and H ₂ SO ₄ | a. diagnostic b. quantitative | 289 |
| 4 | Simple sugars | unbound Cellulose Powder MN-300 | a. EtOAc-pyridine-H ₂ O (2:1:2) b. phenol, equilibrated with 1% NH ₄ OH c. 2-propanol-pyridine-HOAc-H ₂ O (80:80:10:4) | anisidine phthalate | diagnostic | 350 |
| 5 | Simple sugars | Silica Gel G | n-PrOH-conc. NH ₄ OH-H ₂ O (6:2:1) | a. ninhydrin b. aniline hydrogen phthalate | a. diagnostic b. preparative c. noted sugar amination during development | 439 |
| 6 | Simple sugars | a. Silica Gel G b. Silica Gel G buffered with boric acid | a. PrOH-EtOAc-H ₂ O (3:2:1) b. BuOH-acetone-H ₂ O (4:5:1) c. PrOH-EtOAc-H ₂ O (7:2:1) d. methyl ethyl ketone-HOAc-H ₂ O (3:1:1) e. Sol. b with 5% NH ₄ OH f. EtOAc-2-PrOH-H ₂ O (65:24:12) g. Kieselguhr G buffered with 0.02 N NaOAc | naphthoresorcinol and H ₂ PO ₄ | a. diagnostic b. used wedge-shaped chromatograms | 307 |

| | | | | | | |
|----|--|--|---|---|--|-----|
| 7 | Glucose and trehalose | Silica Gel G | <i>sec</i> -BuOH saturated with H ₂ O | NaIO ₄ -KMnO ₄ | a. diagnostic b. could not separate more complex mixtures | 454 |
| 8 | Ten sugar acetates and eight inositol acetates | Silica Gel G | benzene with 2-10% MeOH | a. H ₂ O b. ferric hydroxamate reagent | a. diagnostic b. preparative c. multiple development | 398 |
| 9 | Sugar acetates | starch-bound acid | silicic a. EtOAc-benzene (3:7) b. MeOH-benzene (2:98) | silver nitrate-NH ₃ -NaOMe | diagnostic | 75 |
| | Sugar benzoates | | c. EtOAc-benzene (3:97)(4:6) | naphthoresorcinol-H ₃ PO ₄ | diagnostic | 308 |
| 10 | Partially methylated sugars, isopropylidene sugars and other derivatives | a. Silica Gel G b. Silica Gel G with boric acid | a. BuOH-acetone-H ₂ O (4:5:1) b. methyl ethyl ketone-HOAc-H ₂ O (3:1:1) c. pet. ether | naphthoresorcinol-H ₃ PO ₄ | a. diagnostic b. quantitative | 120 |
| 11 | Sucrose and raffinose | Starch-bound silica gel | a. 2-PtOH-toluene-EtOH-H ₂ O (10:2:5:2.5) b. toluene-EtOAc-EtOH (95%) (10:5:5) | naphthoresorcinol-H ₃ PO ₄ 2'-7'-dichlorofluorescein | a. diagnostic b. quantitative | 441 |
| | Sucrose esters | | | anisaldoxyde-H ₂ SO ₄ -HOAc | diagnostic | 5 |
| 12 | Maltodoligosaccharides up to ten glucose units | Kieselguhr G | a. BuOH-pyridine-H ₂ O (65:20:15) and six other combinations b. BuOH-EtOH-H ₂ O (50:30:20) c. BuOH-2,6-lutidine-H ₂ O (60:30:10) | | | 203 |
| 13 | Aldose 2,4-DNPH's | a. Aluminum Oxide G b. Silica Gel G | a. toluene-EtOAc (1:1) b. toluene-EtOAc (3:1) (1:1) | a. colored compounds b. NaOH | diagnostic | 7 |
| 14 | Sugar derivatives | alumina | | | | |
| 15 | <i>p</i> -Bromophenoxylosazones of xylose derivatives | plaster-of-Paris-bound silica gel | benzene-MeOH (9:1) | colored compounds | | |

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TABLE 11.4—*Cont.*

| Compound | Absorbent | Developer | Visualization | Comments | Ref. |
|---|--|---|--|-----------------------------|------|
| 16 Cardiac glycosides | unbound, deactivated (H ₂ O or HOAc) silica gel | benzene-EtOH (3:1) saturated with H ₂ O | a. trichloroacetic acid-chloramine-T b. SbCl ₃ and acetic anhydride in CHCl ₃ | diagnostic | 322 |
| 17 Cardiac glycosides | Silica Gel G | EtOAc-pyridine-H ₂ O (5:1:4), upper phase | SbCl ₃ in CHCl ₃ | diagnostic | 392 |
| 18 Cardiac glycosides | Silica Gel G | a. CHCl ₃ -MeOH (9:1) b. CHCl ₃ -acetone (65:35) | H ₂ SO ₄ and acetic anhydride | diagnostic | 378 |
| 19 Glycosides of <i>atracytis gummifera</i> | Silica Gel G | a. BuOH-MeOH-H ₂ O (8:1:1) b. PrOH-xylene-H ₂ O (7:2:1) | conc. H ₂ SO ₄ | diagnostic | 100 |
| 20 k-Strophanthin | Silica Gel G | BuOH-MeOH-formamide (17:2:1) | a. SbCl ₃ in CHCl ₃ b. trichloroacetic acid-chloramine-T c. Kedde reagent d. conc. H ₂ SO ₄ | diagnostic | 223 |
| 21 Flavone and coumarin glycosides | Silica Gel G | EtOAc-methyl ethyl ketone-formic acid-H ₂ O (5:3:1:1) | diphenylboric acid, β -aminoethyl ester | diagnostic | 379 |
| 22 Flavone glycosides | starch-bound silica gel | a. EtOAc-CHCl ₃ (95:5) b. EtOAc-EtOH (95:5) c. 3-methyl-1-butanol-hexane-HOAc-H ₂ O (3:1:3:3) | a. AlCl ₃ b. uronium acetate | diagnostic | 286 |
| 23 Flavanol glycosides | Silica Gel G | a. HOAc-formic acid-H ₂ O-MeOH (10:2:2:1) | NH ₃ vapor | diagnostic and quantitative | 126 |
| Cardiac glycosides | — | b. methyl ethyl ketone-volucene-H ₂ O-HOAc-MeOH (40:5:3:1:2:5) | SbCl ₃ in CHCl ₃ | — | — |

| | | | | | | |
|----|-------------------------------------|--|--|--|--|-----|
| 24 | Rutin | a. unbound polyamide powder | a. MeOH b. MeOH-H ₂ O (8:2) (6:4) | a. diazotized sulfanilic acid b. U.V. | diagnostic | 74 |
| | | b. Silica Gel G | c. BuOH-HOAc (9:1) saturated with H ₂ O | <i>p</i> -dimethylaminobenzaldehyde in H ₂ SO ₄ | a. diagnostic b. on microscope slides | 437 |
| 25 | Brain gangliosidic sub- stances | Silica Gel G | PrOH-1 N NH ₄ OH-H ₂ O (6:2:1) | | a. diagnostic b. monitored column | 440 |
| 26 | Catalpa glucosides | Silica Gel G | a. MeOH b. MeOH-EtOEt mixtures | conc. H ₂ SO ₄ | diagnostic | 39 |
| 27 | Anthocyanins and their aglucones | polyacrylonitrile-“Per- lon” (7:1) buffered with K ₂ HPO ₄ | 1-pentanol-1-propanol-HOAc-H ₂ O (3:2:2:1) plus 2 parts 1-heptanol | | diagnostic | 36 |
| 28 | Misc. glycosides | | | | | 189 |

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TABLE 11.5. CARBOXYLIC ACIDS AND THEIR DERIVATIVES (EXCLUSIVE OF FATTY ACIDS)

| | Compound | Absorbent | Developer | Visualization | Comments | Ref. |
|---|---|---|---|--|----------------------------------|-------------------|
| 1 | 2,6-Dimethyl-2,6-octadienoic acid (Hildebrandt's acid) | Silica Gel G | CHCl ₃ :HOAc (97:3) | KMnO ₄ | diagnostic | 428 |
| 2 | Dicarboxylic acids | Silica Gel G | a. benzene-MeOH-HOAc (45:8:4) b. benzene-dioxane-HOAc (90:25:4) | bromphenol blue acidified with citric acid | diagnostic | 302 |
| 3 | Carboxylic acids (as ammonium salts) | Silica Gel G | a. EtOH(95%)-H ₂ O-NH ₄ OH(25% NH ₃)(100:12:16) | brom cresol green | diagnostic | 42 |
| 4 | Methoxy-piperonylic acids | Silica Gel G | EtOAc-hexane-HOAc (50:50:0.5) | chromotropic acid-H ₂ SO ₄ | diagnostic | 32 |
| 5 | Phenolic acids | see "Phenols, Alcohols, etc.", No. 9 and No. 12 | | | | 225 288 290 |
| 6 | Aromatic acids | a. gelatin-bound Silica Gel G "Perlon" (0.5:7:4) b. polyacryloylurethane "Perlon" (8:4) | a. <i>n</i> -amyl alcohol- <i>n</i> -BuOH-EtOAc-HOAc-H ₂ O (60:20:30:10:5) b. <i>n</i> -octane-EtOAc- <i>n</i> -amyl alcohol-EtOEt-formic acid (40:50:15:15:10) | U.V. pentane-HOAc (88:12) | diagnostic | 36 |
| 7 | <i>p</i> -Hydroxybenzoic acid esters | Silica Gel G with fluorescent material | | U.V. | quantitative | 118 |
| 8 | Phthalates, phosphates and other esters as plasticizers | see "Miscellaneous," No. 14 | | | | 293 |
| 9 | Gallic acid and its esters | unbound powder | polyamide a. MeOH b. EtOH c. EtOEt d. CCl ₄ -MeOH (7:3) e. six others | | a. diagnostic b. antioxidants | 69 |

| | | | | | |
|----|---|---|---|--|------------|
| 10 | Lactones, lactams and thiolactones | Silica Gel G | a. isopropyl ether b. isopropyl ether-EtOAc (80:20) c. isopropyl ether- <i>iso</i> -octane (20:80) (60:40) | a. NaOH-hydroxylamine-HOAc-FeCl ₃ consecutively b. Dragendorff's reagent | 207 |
| 11 | Dicarboxylic acids (from oxalic to sebacic) and carbocyclic acids | Kieselguhr G-Polyethylene glycol (M-1000) (2:1) | isopropyl ether-formic acid-H ₂ O (90:7:3) | bromcresol purple | 200 201 |

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TABLE 11.6. DRUGS AND PHARMACEUTICALS

| | Compound | Adsorbent | Developer | Visualization | Comments | Ref. |
|----|---|--|--|---|--------------------------------------|------------|
| 1 | Caffeine, theobromine and theophylline | Silica Gel G | a. EtOAc-MeOH-12 N HCl (18:2:0.05) b. EtOAc-MeOH-HOAc (8:1:1) c. CHCl ₃ -MeOH (19:1) | sublimation from layer | a. diagnostic b. preparative | 10 |
| 2 | Meprobamate | starch-bound silicic acid | EtOH-cyclohexane (15:85) | conc. H ₂ SO ₄ | a. diagnostic b. quantitative | 102 240 |
| 3 | Barbiturates | see "Alkaloids," No. 6 | | | | 226 227 |
| 4 | Nitrogen-containing drugs | Silica Gel G | a. organic b. buffer solutions depending upon drug solutions | a. I ₂ in CHCl ₃ b. potassium iodoplantate | a. diagnostic b. semiquantitative | 279 |
| 5 | Narcotics in forensic chemistry | see "Miscellaneous," No. 19 | | | | 92 |
| 6 | Alkaloidal drugs in urine | see "Alkaloids," No. 7 | | | | 57 |
| 7 | Urinary metabolites of psychotropic drugs | Silica Gel G | BuOH-HOAc-H ₂ O (88:5:7) (65:15:20) | conc. H ₂ SO ₄ | diagnostic | 101 |
| 8 | Chloramphenicol | aluminum oxide | | | monitored synthesis | 217 |
| 9 | Penicillin preparations | Silica Gel G | a. acetone-MeOH (1:1) b. 2-PrOH-MeOH (30:70) | sodium azide-I ₂ solution | diagnostic | 103 |
| 10 | Tetracyclines, penicillins and rifomycins | a. Silica Gel G b. Silica Gel G buffered to pH 4.5 or 4.2 | a. aqueous citric acid solutions b. pure alcohols c. many others | a. HCl and others b. microbiological assay | diagnostic | 275 276 |
| 11 | Antibiotics (macrolides) | Silica Gel G | acetone-H ₂ O (98:2) | microbiological assay | diagnostic | 33 |

| | | | | | | |
|----|-----------------------------------|---|---|--|--|------------|
| 12 | Sulfonamides | a. Silica Gel G b. Silica Gel G containing fluorescein | CHCl ₃ -EtOH-heptane (1:1:1) plus 1.0 to 1.8% H ₂ O | a. HCl-NaNO ₂ -N(1-naphthyl) ethylenediamine b. U.V. | diagnostic | 196 |
| 13 | Sulfonamides | Silica Gel G | CHCl ₃ -EtOH-heptane (1:1:1) | <i>p</i> -dimethylaminobenzaldehyde, acidified | diagnostic | 452 |
| 14 | Sulfonamide, radioactive | Silica Gel G | BuOH sat. with conc. NH ₃ | radioactive method | a. diagnostic b. quantitative c. preparative | 349 |
| 15 | Misc. constituents of plant drugs | | | | | 379 |
| 16 | Pharmaceutical applications | | | | review | 122 340 |

Thin-Layer Chromatography

TABLE 11.7. DYES

| | Compound | Adsorbent | Developer | Visualization | Comments | Ref. |
|---|--|--------------------------------------|---|--|---|-------------------|
| 1 | Food dyes | unbound aluminum oxide | a. $\text{Ba(OH}\cdot\text{EtOH}\cdot\text{H}_2\text{O}$ (90:10:10) (80:20:10)(70:30:30) (60:40:40)(50:50:50) | colored compounds | a. diagnostic b. spot made alkaline before chromatography c. circular | 266 269 306 |
| 2 | Food dyes | Cellulose Powder MN 300 G and MN 300 | a. aqueous sodium citrate (2.5%) NH_4OH (25%) (4:1) b. $\text{P(OH-EtOAc-H}_2\text{O}$ (6:1:3) c. $t\text{-BuOH}$ -propanoic acid- H_2O (50:12:38) with 0.4% KCl | colored compounds | diagnostic | 451 |
| 3 | Food dyes | unbound aluminum oxide | a. MeOH b. EtOH c. CHCl_3 d. CCl_4 e. EtOEt f. seven others | colored compounds | a. diagnostic b. quantitative | 73 |
| 4 | Food dyes | Silica Gel G | a. CHCl_3 -acetic anhydride (75:2) b. benzene c. methyl ethyl ketone- HOAc-MeOH (40:5:5) | SiO_2Cl in CHCl_3 | diagnostic | 257 |
| 5 | Carotenoid dyes | see "Vitamins," No. 2 | | | | |
| 6 | Carotenoid dyes, synthetic | calcium hydroxide-Silica Gel G (6:1) | pet. ether-benzene (2:3) | colored compounds | a. diagnostic b. monitored synthesis | 166 |
| 7 | Dyes in gasoline | Silica Gel G | benzene | colored compounds | a. diagnostic b. to catch gasoline thieves | 139 |
| 8 | Test mixture dyes: butter yellow, Sudan red III and indophenol | see "Miscellaneous," No. 16 | | | | 381 |

TABLE II.3. ESSENTIAL OILS

| | Compound | Absorbent | Developer | Visualization | Comments | Ref. |
|---|------------------------------|---|---|--|--|-------------------|
| 1 | Essential oils of Argentina | starch-bound silicic acid containing fluorescent material | a. hexane b. pet. ether-Et ₂ O-Ac mixtures | a. U.V. b. fluorescein-Br ₂ c. bromoresol green d. 2,4-DNPH e. conc. H ₂ SO ₄ | diagnostic | 105 106 107 |
| 2 | Thirteen essences | starch-bound silicic acid | a. hexane b. benzene-ethanol mixtures | a. SbCl ₃ in CHCl ₃ b. 2,4-DNPH c. KMnO ₄ d. <i>p</i> -dimethylaminobenzaldehyde-H ₂ SO ₄ | diagnostic | 287 |
| 3 | Misc. essential oils | Silica Gel G | benzene-CHCl ₃ (1:1) | SbCl ₃ in CHCl ₃ | a. diagnostic b. used with gas chromatography | 382 |
| 4 | Perfume industry | Silica Gel G | a. hexane b. benzene c. CH ₂ Cl ₂ d. CHCl ₃ | a. SbCl ₃ in CHCl ₃ b. SbCl ₅ in CCl ₄ c. conc. H ₂ SO ₄ d. vanillin and other aldehydes in H ₂ SO ₄ e. fluorescein-Br ₂ f. 2,4-DNPH g. <i>o</i> -dianisidine | a. diagnostic b. circular c. in shaped areas | 368 |
| | a. Essential oils | — | — | — | — | |
| | b. Single essences | — | — | — | — | |
| | c. Balsams and resins | — | — | — | — | |
| | d. Tar and tar products | — | — | — | — | |
| | e. Phenols and phenol ethers | — | — | — | — | |
| | f. Wool wax and derivatives | — | — | g. CHCl ₃ -MeOH mixtures | h. SbCl ₅ in CCl ₄ i. acetic anhydride-H ₂ SO ₄ | |
| | g. Azulenes | — | — | h. hexane i. benzene | j. chloranil in CHCl ₃ k. Br ₂ in CHCl ₃ | |
| 5 | Misc. essential oils | see "Terpenoids," No. 1 | — | — | — | 190 |

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TABLE 11.8—Cont.

| | Compound | Adsorbent | Developer | Visualization | Comments | Ref. |
|----|--------------------------------|---|---|---|---|------------|
| 6 | a. Misc. essential oils | starch-bound silicic acid containing fluorescent material (Rhodamine 6 G) | a. hexane b. hexane-EtOAc mixtures | a. U.V. b. 2,4-DNPH | diagnostic | 323 |
| | b. Oils of <i>Mentha</i> genus | — | — | — | — | 324 |
| | c. Mint oils | — | — | — | quantitative | 326,327 |
| 7 | Oils of Japanese mint | starch-bound silicic acid | hexane-EtOAc (85:15) | vanillin in H ₂ SO ₄ | diagnostic | 325 |
| 8 | Oils of <i>Mentha piperita</i> | Silica Gel G | hexane | a. SbCl ₃ in CrCl ₃ b. transparency method | quant. assay of menthofuran | 167 168 |
| 9 | Oils of peppermint | — | — | — | — | 297 |
| 10 | Oils of <i>Oleum menthae</i> | Silica Gel G | a. benzene-EtOAc (95:5) b. hexane | a. vanillin in H ₂ SO ₄ b. SbCl ₃ in CH ₂ Cl ₂ c. SbCl ₅ in CCl ₄ d. fluorescein-Br ₂ e. 2,4-DNPH f. KI in HOAc with Na ₂ SO ₃ and followed by starch g. HNO ₃ -HOAc (1:300) | diagnostic | 171 |
| 11 | Oils of peppermint | plaster-of-Paris-bound silicic acid | “Skellysolve” B-EtOAc mixtures, up to 10% EtOAc | a. transmitted light b. U.V. c. trichloroacetic acid in CHCl ₃ d. KMnO ₄ in H ₂ SO ₄ e. Rhodamine B | a. diagnostic b. study site and mode of terpene formation in plant c. preparative | 19 20 |
| 12 | Menthol in peppermint oil | alumina-silicate | — | — | quantitative | 133 |

| | | | | | | |
|----|---|--|---|---|--|-----|
| 13 | Oils of Eucalyptus | starch-bound silica gel | hexane-EtOAc (85:15) | conc. H ₂ SO ₄ | a. diagnostic b. circular | 313 |
| 14 | Oils of Eucalyptus | starch-bound magnesol | | | a. diagnostic b. circular | 51 |
| 15 | Oils of <i>Strobilanthes</i> <i>Limifolia</i> | a. silica gel ("Celite")-starch (16:4:1) b. plaster-of-Paris-bound silica gel | hexane-EtOAc mixtures | | a. diagnostic b. monitored column | 58 |
| 16 | Oil of jasmine | see "Terpenoids," No. 13 | | | | |
| 17 | Azulene of camphor blue oil | see "Terpenoids," No. 14 | | | | |
| 18 | Oil of <i>Pogostemon patchouli</i> Pell | starch-bound silicic acid | a. hexane-EtOAc (85:15) b. solution a plus 5% CHCl ₃ | conc. H ₂ SO ₄ | a. diagnostic b. used "chromato-bars" | 108 |
| 19 | Oils of <i>Aniba montana</i> L. | starch-bound silicic acid and <i>terra siccata</i> | CHCl ₃ | conc. H ₂ SO ₄ | diagnostic | 124 |
| 20 | Oils of <i>grapefruit</i> | a. starch-bound silicic acid b. plaster-of-Paris-bound silicic acid | a. hexane b. 2,2-dimethylbutane c. cyclohexane d. methylcyclohexane e. isopentane | a. fluorescein-Br ₂ b. HNO ₃ -H ₂ SO ₄ (5:95) | a. diagnostic b. predicted solvents for column work | 131 |
| b. | Oxygenated compounds | — | — | f. hexane-EtOAc mixtures g. EtOAc-CHCl ₃ (alcohol free) mixtures h. EtOAc-benzene (15:85) i. hexane-nitropropane j. isopropyl ether-hexane (50:50) | | |
| 21 | Oils of orange juice | starch-bound silicic acid | | hexane-EtOAc (83:15) plus a variety of others | diagnostic | 192 |
| | | | | a. fluorescein-Br ₂ b. 2,4-DNPH c. o-dianisidine d. conc. sulfuric-nitric acid | | |

Thin-Layer Chromatography

TABLE 11.8—*Cont.*

| Compound | Absorbent | Developer | Visualization | Comments | Ref. |
|--|--|--|---|---|-------------------|
| 22 Oils of lemon a. Menthyl salicylates | starch-bound silicic acid with phosphors | a. EtOEt-pet. ether mixtures | a. U.V. | a. quantitative b. descending tech. | 383 |
| b. Coumarins | — | b. hexane-EtOAc (85:15) | b. 2,4-DNP-H c. o-dianisidine in glacial HOAc d. U.V. | c. monitored column d. diagnostic | 387 388 417 |
| c. Hydrocarbons | — | c. heptane to develop and elute hydrocarbons from end of layer | c. Fluorescein-Br ₂ | e. quant. assay of hydrocarbons by gas chromatography | 385 |
| d. Citral | — | d. hexane-EtOAc (85:15) | f. fluorescein-Br ₂ , viewed under U.V. | f. diagnostic | 386 |
| e. Chalcones | — | e. hexane-EtOAc (75:25) f. EtOEt-pet. ether (65:35) | g. HCl gas h. U.V. | g. diagnostic | 384 |
| 23 Oils of sage | Silica Gel G | benzene | vanillin in H ₂ SO ₄ | diagnostic | 49 |
| 25 Volatile oil of grafted umbelliferous plants | — | — | — | diagnostic | 198 199 |
| 26 Oils of <i>Curcumina</i> | Silica Gel G | benzene-CH ₂ Cl-MeOH (200:100:3) | SbCl ₃ in CH ₂ Cl ₂ | diagnostic | 445 |
| 27 Oils of <i>Anethus calamus</i> | Silica Gel G | benzene | — | a. diagnostic b. to relate plants | 453 |
| 28 Oils of <i>coriander lavender</i> and muscat sage | — | — | — | — | 298 |

| | | | | | | |
|----|--|---------------------------------|----------------------|--|------------|-----|
| 29 | Oils of <i>Oleinum rosmarinii</i> , <i>O. Lavandulae</i> and <i>O. roseae</i> | Silica Gel G | benzene-EtOAc (95:5) | a. SbCl ₃ in CHCl ₃ b. SbCl ₃ in CCl ₄ c. conc. H ₂ SO ₄ | diagnostic | 172 |
| 30 | 2,4-DNPH's of aldehydes and ketones from essential oils | see "Aldehydes, etc." No. 14 | | | | 209 |
| 31 | Removal of hydrocarbons from essential oils | see "Terpenoids," No. 1 | | | | 190 |

Thin-Layer Chromatography

TABLE 11.9. FLAVONOIDS AND COUMARINS

| | Compound | Adsorbent | Developer | Visualization | Comments | Ref. |
|---|-----------------------------------|--|---|--|--------------------------------------|------------------|
| 1 | Isoflavones | starch-bound silicic acid with phosphors | a. EtOAc-Skellysolve B (3:1) (1:1) b. acetone-EtOAc-Skellysolve B (4:3:3) c. EtOH-CHCl ₃ (1:3)(1:1) | U.V. | a. diagnostic b. preparative | 132 |
| 2 | Flavonoids | unbound polyamide powder (Silion) | a. MeOH-H ₂ O (80:20)(60:40) b. MeOH | U.V. | diagnostic | 71 74 |
| 3 | Flavonoids and coumarins | Silica Gel G-NaOAc | toluene-ethyl formate-formic acid (5:4:1) | diphenylboric acid, β -aminoethyl ester | diagnostic | 379 |
| 4 | Flavonoids and coumarins of lemon | starch-bound silicic acid with phosphors | CHCl ₃ -acetone mixtures | U.V. | a. diagnostic b. monitored column | 162 |
| 5 | Coumarins | Silica Gel G | a. pet. ether-EtOAc (2:1) b. hexane-EtOAc (5:2) | KOH and diazotized sulfanilic acid | diagnostic | 396 |
| 6 | Coumarins in lemon juice | starch-bound silicic acid with phosphors | hexane-EtOAc (75:25) | U.V. | preparative | 31 |
| 7 | Coumestrol in plants | starch-bound silicic acid with phosphors | a. CHCl ₃ -EtOEt (1:1) b. EtOAc-“Skellysolve” B (3:1) c. acetone-“Skellysolve” B (1:3) d. EtOEt-“Skellysolve” B (7:3) | U.V. | a. diagnostic b. quantitative | 222 224 34 |

TABLE 11.10. INORGANIC AND METALORGANIC COMPOUNDS

| | Compound | Adsorbent | Developer | Visualization | Comments | Ref. |
|---|--|---|--|--|---|------|
| 1 | Fe ⁺² , Zn ⁺² , Be ⁺² , Ni ⁺² , and others | starch-bound alumina; "Celite" | a. H ₂ O b. dilute HCl c. organic liquids | a. I ₂ b. U.V. | a. diagnostic b. circular on microscope slides | 246 |
| 2 | a. Copper family-Cu ⁺² , Cd ⁺² , Bi ⁺³ , Pb ⁺² , Hg ⁺² b. Sulfide group-Zn ⁺² , Co ⁺² , Fe ⁺² , Ni ⁺² , Mn ⁺² , Cr ⁺³ , Al ⁺³ | purified Silica Gel G | a. BuOH-1.5 N HCl-2,5-hexanedione (100:20:0.5) b. acetone-conc. HCl-2,5-hexanedione (100:1:0.5) | a. KI b. NH ₃ and H ₂ S c. NH ₃ d. β-hydroxyquinoline e. U.V. | diagnostic theory | 359 |
| 3 | U ⁺⁶ , Ga ⁺³ and Al ⁺³ | purified Silica Gel G | a. EtOAc (sat. with H ₂ O)-tributyl phosphate (50:2) b. acetone-conc. HCl (100:0.5) | a. pyridyl-azo-naphthalol b. β-hydroxyquinoline c. U.V. | diagnostic | 360 |
| 4 | Alkali metals-Na ⁺¹ , Li ⁺¹ , K ⁺¹ , Mg ⁺² | purified Silica Gel G | EtOH-HOAc (100:1) | acid violet | diagnostic | 358 |
| 5 | Halogens-F ⁻¹ , Cl ⁻¹ , Br ⁻¹ , I ⁻¹ | purified Silica Gel G | acetone-BuOH-conc. NH ₄ OH-H ₂ O (65:20:10:5) | a. brom cresol purple and NH ₃ b. ammonical AgNO ₃ and fluorescein | diagnostic | 357 |
| 6 | Various phosphates | purified Silica Gel G | MeOH-conc. NH ₄ OH-10% trichloroacetic acid and H ₂ O (50:15:5:30) | ammonium molybdate followed by SnCl ₂ and HCl | diagnostic | 355 |
| 7 | K ⁺¹ and Mg ⁺² | Silica Gel G | EtOH-MeOH (1:1) plus HOAc | acid violet 6BN | quantitative | 315 |
| 8 | Radioactive halogens | "Dowex 1" ¹² Cellulose Powder MN 300 G (1:1) | 1 M aqueous NaNO ₃ | radioactivity | diagnostic | 29 |

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TABLE 11.9. FLAVONOIDS AND COUMARINS

| | Compound | Adsorbent | Developer | Visualization | Comments | Ref. |
|---|------------------------------------|--|--|---|---|------------------|
| 1 | Isoflavones | starch-bound silicic acid with phosphors | a. EtOAc-Skellysolve B (3:1) (1:1) b. acetone-EtOAc-Skellysolve B (4:3:3) c. EtOH-CHCl ₃ (1:3)(1:1) | U.V. | a. diagnostic b. preparative | 132 |
| 2 | Flavonoids | unbound polyamide powder (Silon) | a. MeOH-H ₂ O (80:20)(60:40) b. MeOH | U.V. | diagnostic | 71 74 |
| 3 | Flavonoids and coumarins | Silica Gel G-NaOAc | toluene-ethyl formate-formic acid (5:4:1) | diphenylboric acid, β -aminomethyl ester | diagnostic | 379 |
| 4 | Flavonoids and coumarins of lemon. | starch-bound silicic acid with phosphors | CHCl ₃ -acetone mixtures | U.V. | a. diagnostic b. monitored column | 162 |
| 5 | Coumarins | Silica Gel G | a. pet. ether-EtOAc (2:1) b. hexane-EtOAc (5:2) | KOH and diazotized sulfanilic acid | diagnostic | 396 |
| 6 | Coumarins in lemon juice | starch-bound silicic acid with phosphors | hexane-EtOAc (75:25) | U.V. | preparative | 31 |
| 7 | Coumestrol in plants | starch-bound silicic acid with phosphors | a. CHCl ₃ -EtOEt (1:1) b. EtOAc-Skellysolve ² B (3:1) c. acetone-Skellysolve ² B (1:3) d. EtOEt-Skellysolve ² B (7:3) | U.V. | a. diagnostic b. quantitative d. EtOEt-Skellysolve ² B (7:3) | 222 224 34 |

TABLE 11.10. INORGANIC AND METALORGANIC COMPOUNDS

| | Compound | Adsorbent | Developer | Visualization | Comments | Ref. |
|---|--|---|--|---|---|------------|
| 1 | Fe^{+2} , Zn^{+2} , Be^{+2} , Ni^{+2} , and others | starch-bound alumina, ^a "Celite" | a. H_2O b. dilute HCl c. organic liquids | a. I_2 b. U.V. | a. diagnostic b. circular on microscope slides | 246 |
| 2 | a. Copper family: Cu^{+2} , Cd^{+2} , Bi^{+3} , Pb^{+2} , Hg^{+2} b. Sulfide group: Zn^{+2} , Co^{+2} , Fe^{+3} , Ni^{+2} , Mn^{+2} , Cr^{+3} , Al^{+3} | purified Silica Gel G | a. $\text{BuOH}-1.5\text{N HCl}-2.5\text{-hexanone}$ (100:20:0.5) b. acetone-conc. HCl -2.5-hexanone (100:1:0.5) | a. KI b. NH_3 and H_2S c. NH_3 d. 8-hydroxyquinoline e. U.V. | diagnostic theory | 359 356 |
| 3 | U^{+6} , Ga^{+3} and Al^{+3} | purified Silica Gel G | a. EtOAc (sat. with H_2O)-tributyl phosphate (50:2) b. acetone-conc. HCl (100:0.5) | a. pyridyl-azo-naphthal b. 8-hydroxyquinoline c. U.V. | diagnostic | 360 |
| 4 | Alkali metals-Na ⁺ , Li ⁺ , K^{+1} , Mg^{+2} | purified Silica Gel G | EtOH-HOAc (100:1) | acid violet | diagnostic | 358 |
| 5 | Halogens-F ⁻¹ , Cl ⁻¹ , Br ⁻¹ , I ⁻¹ | purified Silica Gel G | acetone-BuOH-conc. NH_4OH - H_2O (65:20:10:5) | a. brom cresol purple and NH_3 b. ammonical AgNO_3 and fluorescein | diagnostic | 357 |
| 6 | Various phosphates | purified Silica Gel G | MeOH -conc. NH_4OH -10% trichloroacetic acid and H_2O (50:15:5:30) | ammonium molybdate followed by SnCl_4 and HCl | diagnostic | 355 |
| 7 | K^{+1} and Mg^{+2} | Silica Gel G | EtOH-MeOH (1:1) plus HOAc | acid violet 6BN | quantitative | 315 |
| 8 | Radioactive halogens | "Dowex 1" ^b -Cellulose Powder MN 300 G (1:1) | 1 M aqueous NaNO_3 | radioactivity | diagnostic | 29 |

TABLE II.10—*Cont.*

| Compound | Adsorbent | Developer | Visualization | Comments | Ref. |
|--|---|--|--|------------|------|
| 9 Twenty-four ferrocene derivatives | Silica Gel G | a. benzene b. benzene-EtOH (30:1)(15:1) c. propylene glycol-MeOH (1:1) d. propylene glycol-chlorobenzene-MeOH (1:1:1) | a. Br ₂ b. NaIO ₄ | diagnostic | 345 |
| 10 Chromium and cobalt complexes of <i>o</i> -hydroxy- <i>o</i> -carboxyazo dyes | Aluminum Oxide Fluka | MeOH | colored compounds | diagnostic | 342 |
| 11 Organo-tin stabilizers in polyvinyl chloride | Silica Gel G containing "Komplexon III" | H ₂ O-BuOH-EtOH-HOAc (10:5:5:0.15) | Diphenylthiocarbazone | diagnostic | 411 |

TABLE 11.11. INSECTICIDES

| | Compound | Adsorbent | Developer | Visualization | Comments | Ref. |
|---|---|------------------------------------|--|---|---|------|
| 1 | a. Thiophosphate esters b. Chlorinated compounds | Silica Gel D5 Aluminum Oxide D5 | hexane-acetone (4:1) hexane | PdCl ₂ in dilute HCl N,N-dimethyl- <i>p</i> -phenylenediamine hydrochloride in sodium methylate | a. diagnostic b. in forensic chemistry | 23 |
| 2 | Dichlorophene and hexachlorophene | starch-bound silicic acid | heptane saturated with HOAc | FeCl ₃ -K ₃ Fe(CN) ₆ | a. diagnostic b. quantitative | 43 |
| 3 | Thiophosphate esters | starch-bound aluminum oxide | heptane-acetone (10:1) | H ₂ IO ₆ -HClO ₄ -V ₂ O ₅ | diagnostic | 304 |
| 4 | Pyrethrins | plaster-of-Paris-bound silica gel | EtOAc-hexane (20:80) | fluorescein-Br ₂ | diagnostic | 365 |
| 5 | Pyrethrins and their peroxides | Silica Gel G | a. benzene-methyl ethyl ketone (90:10) b. benzene-EtOAc (85:15) c. CCl ₄ -EtOAc (80:20) d. hexane-methyl ethyl ketone (80:20) e. hexane-EtOAc (75:25) | SbCl ₃ in CHCl ₃ | a. diagnostic b. reaction on layer | 371 |
| 6 | Pentachlorophenol | Silica Gel G-oxalic acid | CHCl ₃ | U.V. | a. diagnostic b. quantitative | 83 |
| 7 | Chlorinated organic pesticides | | | | | 455 |
| 8 | Chlorinated hydrocarbons with insecticidal properties | | | | | 300 |

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TABLE 11.12. LIPIDS—CLASS SEPARATION

| | Compound | Absorbent | Developer | Visualization | Comments | Ref. |
|---|-----------------------------|--|--|---|--|------|
| 1 | Misc. lipids | Silica Gel G | a. EtOEt b. isopropyl ether c. isopropyl ether-HOAc (98:5:1.5) | Phosphomolybdic acid | Class separation prior to analysis by reversed phase technique | 182 |
| 2 | Misc. lipids | Silica Gel G | pet. ether-EtOEt-HOAc (90:10:1) | 2',7'-dichlorofluorescein | Class separation prior to analysis by reversed phase technique | 230 |
| 3 | Misc. lipids | | | | monitored column | 150 |
| 4 | Radioactive lipids | silicic acid | pet. ether-EtOEt (92:8) | a. radioactivity b. α -cyclodextrin-I ₂ c. I ₂ | class separation | 231 |
| 5 | Industrial aliphatic lipids | a. Silica Gel G b. benzene sat. at 20° with 1 N NH ₄ OH c. CHCl ₃ (sat. with 1 N NH ₄ OH)-MeOH (97:3) d. acetone-14 N NH ₄ OH (90:10) | a. pet. ether-benzene (95:5) b. 2',7'-dichlorofluorescein c. K ₂ Cr ₂ O ₇ in H ₂ SO ₄ | a. I ₂ b. 2',7'-dichlorofluorescein | diagnostic | 234 |
| | b. Silica Gel G | e. CHCl ₃ -(MeOH-0.1 N H ₂ SO ₄ , 95:5)(97:3)(80:20) | | | | |
| 6 | Misc. fats, oils and waxes | Silica Gel G | a. pet. ether-EtOEt-HOAc (90:10:1)(70:30:2)(70:30:1) b. hexane-EtOEt (95:5) | a. I ₂ b. 2',7'-dichlorofluorescein | a. diagnostic b. class separation | 235 |
| 7 | Tissue lipids | Silica Gel G | hexane-EtOEt-HOAc (90:10:1) | 2',7'-dichlorofluorescein | class separation prior to assay by gas chromatography | 236 |
| 8 | Serum lipids | unbound aluminum oxide | a. pet. ether-EtOEt (95:5) b. pet. ether-EtOH (98:2) c. pet. ether-EtOEt-HOAc (94:5:5:0.5) d. heptane-HOAc (98:2) | | a. class separation b. quantitative | 413 |

| | | | | | | |
|----|---|-------------------------------------|--|--|--|------------------|
| 9 | Serum lipids and phospholipids | Plaster-of-Paris-bound silica acid. | a. $\text{CHCl}_3\text{-MeOH-H}_2\text{O}$ (80:25:3) b. pet. ether-EtOEt-H ₂ OAc (90:10:1)(60:40:1) | $2',7'$ -dichlorofluorescein | diagnostic | 423 |
| 10 | Serum lipids | Silica Gel G | a. PrOH-NH ₄ OH (2:1)-1st step b. $\text{CHCl}_3\text{-benzene}$ (3:2) 2nd step c. CCl_4 -reverse | SbCl ₃ in CHCl_3 | a. stepwise technique b. two dimensional c. quantitative | 21 164 438 |
| 11 | Serum, liver and adipose tissue lipids | Silica Gel G | | | | 86 |
| 12 | Plasma lipids | Silica Gel G | $\text{CHCl}_3\text{-MeOH-H}_2\text{O}$ (65:25:4) | a. bromthymol blue-NH ₃ b. ninhydrin c. fuchsin-H ₂ SO ₄ d. hydroxamate reaction | a. diagnostic b. quantitative for phosphates | 134 |
| 13 | Plasma lipids | Silica Gel G | a. pet. ether-diisobutylketone-HOAc (87:13:0.7) b. pet. ether-methyl ethyl ketone-HOAc (95:4:1) | a. 50% H ₂ SO ₄ b. Rhodamine 6 G c. bromthymol blue d. osmium tetroxide | class separation prior to separation by reversed phase technique | 459 |
| 14 | Butter-fat lipid | | | | | 245 |
| 15 | Brain tissue lipids | Silica Gel G | a. $\text{CHCl}_3\text{-MeOH-H}_2\text{O}$ (24:7:1) b. CHCl_3 c. CCl_4 | a. ammonium molybdate-HClO ₄ b. phosphomolybdic acid c. ninhydrin d. Dragendorff's reagent e. diphenylamine | diagnostic on multiple sclerosis patients | 157 |
| 16 | Brain lipid constituents (pure test substances) | Silica Gel G | a. heptane b. CCl_4 c. 1,2-dichloroethane d. CHCl_3 e. 1,2-dichloroethane-MeOH (98:2) f. pet. ether-EtOEt (70:30) g. four others | a. bromthymol blue, buffered b. bromthymol blue c. conc. H ₂ SO ₄ -HOAc (1:1) d. phosphomolybdic acid e. HIO ₄ -Schiff reagent f. aqua regia-ammonium molybdate-vanadyl chloride g. diphenylamine | a. diagnostic b. system for exploring unknown lipids | 174 |

TABLE II.12—*Cont.*

| | Compound | Adsorbent | Developer | Visualization | Comments | Ref. |
|----|------------------------------------|--------------|---------------------------------|------------------------------------|--|------------|
| 17 | Dogfish liver lipids | Silica Gel G | pet. ether-EtOEt-HOAc (90:10:1) | | separated the alkoxy-glycerides class for assay | 229 |
| 18 | Lipids of feces and fecal- iths | Silica Gel G | pet. ether-EtOEt-HOAc (80:20:1) | 50% H ₂ SO ₄ | a. class separation b. semiquantitative assay | 442 |
| 19 | Lipids | | | | review review | 104 232 |

TABLE 11.13. LIPIDS—FATTY ACIDS AND THEIR DERIVATIVES

| | Compound | Absorbent | Developer | Visualization | Comments | Ref. |
|---|--|---|--|---|---|------|
| 1 | Fatty acids | plaster of Paris impregnated with undecane | a. HOAc-CH ₃ CN (1:1) b. solution a with 0.5% Br ₂ | copper acetate-dithiooxamide | separated "critical" pairs after hydrogenation and bromination on layer | 180 |
| 2 | Fatty acids | Kieselguhr G impregnated with undecane | HOAc-CH ₃ CN (1:1) | copper acetate-dithiooxamide on layers "smoked" with dichlorodimethylsilane | diagnostic | 185 |
| 3 | Fatty acids | Kieselguhr G impregnated with undecane | a. HOAc-CH ₃ CN (6:4), 90% sat. with undecane b. HOAc-CH ₃ CN (30:70), 80% sat. with undecane and containing 0.5% Br ₂ | Rhodamine B | separated "critical" pairs after hydrogenation and bromination on layer | 186 |
| 4 | Fatty acids | Silica Gel G impregnated with undecane | a. 96% HOAc b. HOAc-CH ₃ CN (1:1) | phosphomolybdic acid | diagnostic | 182 |
| 5 | Fatty acids of Swiss Pharmacopoeia | see "Lipids-Glycerides", No. 16 | | | | 6 |
| 6 | Keto- and hydroxy-fatty acids and their lactones | Kieselguhr G impregnated with tetradecane or paraffin | a. HOAc-H ₂ O (80:20), 80% sat. with impregnating solution (5% of agent in pet. ether) b. HOAc-H ₂ O (90:10), 75% sat. with impregnating solution | a. Rhodamine B b. phosphomolybdic acid | diagnostic | 181 |
| 7 | Fatty acids of castor oil and oiticica oil | Silica Gel G | pet. ether-EtOEt-HOAc (70:30:1)(70:30:2) | 2',7'-dichlorofluorescein | diagnostic | 235 |
| 8 | Lignoceric, cerebronic and nervonic acids | Silica Gel G | | | diagnostic | 109 |
| 9 | Fatty acid methyl esters | starch-bound silicic acid with phosphors | a. "Skellysolve" F-EtOEt (90:10)(70:30)(50:50) b. benzene-EtOEt (75:25) c. fluorescein-Br ₂ | a. 2',7'-dichlorofluorescein b. U.V. c. fluorescein-Br ₂ | a. diagnostic b. monitored column c. U.V. | 8 |

TABLE 11.13—Cont.

| | Compound | Adsorbent | Developer | Visualization | Comments | Ref. |
|----|----------------------------------|--|---|--|---|------|
| 10 | Fatty acid methyl esters | Silica Gel G impregnated with AgNO_3 | EtOEt-hexane (10:90)(40:60) | 2',7'-dichloro-fluorescein | diagnostic | 260 |
| 11 | Fatty acid methyl esters | Silica Gel G | hexane-EtOEt-HOAc (85:15:1) | 2',7'-dichloro-fluorescein | preparative separation prior to analysis by gas chromatog. | 333 |
| 12 | Fatty acid methyl esters | Silica Gel G | hexane-EtOEt mixtures (up to 30% EtOEt) | a. I ₂ b. 2',7'-dichloro-fluorescein c. 50% H_2SO_4 | a. quantitative b. preparative | 420 |
| 13 | Fatty acid methyl esters | Silica Gel G impregnated with Silicone oil | a. $\text{CH}_3\text{CN}-\text{HOAc}-\text{H}_2\text{O}$ (70:10:25) b. peracetic acid-HOAc-H ₂ O (10:75:15) | a. I ₂ α -cyclodextrin and I ₂ b. solution a plus 1% HOAc | a. diagnostic b. separated sat. esters by using an oxidizing developer | 230 |
| 14 | Fatty acid methyl esters | Silica Gel G | a. pet. ether-EtOEt (80:20) b. solution a plus 1% HOAc | 50% H_2SO_4 | diagnostic | 262 |
| 15 | Epoxy fatty acid methyl esters | Silica Gel G | hexane-EtOEt (95:5)(90:10) | 50% H_2SO_4 | monitored various separation methods | 261 |
| 16 | Fatty acids and esters | silicic acid on the inside of test tubes | a. hexane b. hexane-EtOEt (97:3)(85:15) c. CHCl_3 | I ₂ | diagnostic | 219 |
| 17 | Fatty acid esters of orujo oil | Silica Gel G | hexane-EtOEt (90:10) | 50% H_2SO_4 | a. diagnostic b. monitored column | 422 |
| 18 | Fatty acid esters of cholesterol | a. Silica Gel G b. Silica Gel G impregnated with paraffin | a. tetralin-hexane (25:75)(1:1) b. CCl_4 c. methyl ethyl ketone- CH_3CN (7:3) | phosphomolybdic acid | a. diagnostic b. two-dimension | 184 |

| | | | | | | |
|----|---|----------------------------------|--|--|--|------------|
| 19 | Fatty acids and their cholesterol esters | Silica Gel G | a. PrOH-NH ₂ OH (2:1) b. CHCl ₃ -benzene (3:2) c. CCl ₄ | SbCl ₃ in CHCl ₃ | a. diagnostic b. stepwise technique c. two-dimension | 438 |
| 20 | Cholesterol and its esters | see "Steroids," No 28 and No. 27 | | | | 228 249 |
| 21 | Acetoxymercurymethoxy derivatives of unsaturated fatty acid methyl esters | Silica Gel G | a. pet. ether-EtOEt (4:1) b. PrOH-HOAc (100:1) | s-diphenylcarbazone | isolated unsaturated esters prior to assay by gas chromatography | 177 233 |
| 22 | p-Phenylazophenacyl esters of fatty acids | Silica Gel G | pet. ether-EtOEt (85:15) | colored compounds | diagnostic | 419 |

TABLE 11.14. LIPIDS—GLYCERIDES

| Compound | Adsorbent | Developer | Visualization | Comments | Ref. |
|------------------|---|---|--|--|----------------|
| 1 Glycerides | Silica Gel G impregnated with AgNO_3 | $\text{CHCl}_3\text{-HOAc}$ (99.5:0.5) | dibromo-R-fluorescein | separation according to degree of unsaturation | 16 |
| 2 Glycerides | Silica Gel G | $\text{CHCl}_3\text{-benzene}$ (70:30) | conc. H_2SO_4 | separation of the glyceride fraction from other fractions | 64 |
| 3 Glycerides | Silica Gel G impregnated with undecane | a. $\text{CHCl}_3\text{-MeOH-H}_2\text{O}$ (5:15:1) for diglycerides b. acetone- CH_3CN (7:3) for triglycerides | Rhodamine B | diagnostic | 182 |
| 4 Glycerides | Silica Gel G | a. "Skellysolve" F-EtOE (70:30) b. (10:90) for monoglycerides c. (70:30) for diglycerides d. (90:10) for triglycerides e. (60:40)(35:65)(85:15) | 50% H_2SO_4 | a. quantitative b. quantitative based upon ozonolysis and periodate oxidation | 311 310 |
| 5 Glycerides | plaster-of-Paris-bound silicic acid | gradient elution system of pet. ether-EtOE varied between (9:1) and (4:6). All contained 0.1% HOAc. | 2',7'-dichlorofluorescein | a. diagnostic b. gradient elution | 334 |
| 6 Glycerides | Silica Gel G | a. 1,2-dichloroethane b. CHCl_3 c. 1,2-dichloroethane-MeOH (98:2) d. pet. ether-EtOE (70:30) e. $\text{CHCl}_3\text{-HOAc}$ (96:4) | a. HIO_4 -Schiff reagent b. bromthymol blue | diagnostic | 174 |
| 7 Monoglycerides | hydroxyl-apatite | methyl isobutyl ketone at 10° | phosphomolybdic acid | diagnostic | 155 |
| 8 Triglycerides | Kieselguhr G impregnated with petroleum | acetone- CH_3CN (8:2)(7:4) | a. phosphomolybdic acid b. I ₂ c. α -cyclodextrin-I ₂ | a. diagnostic b. multiple development | 179 |

| | | | | | | |
|----|---|--|---|--|---|-----|
| 9 | Triglycerides | plaster of Paris impregnated with paraffin (b.p. 240-250°) | a. acetone-CH ₃ CN (8:2) b. propanoic acid-CH ₃ CN (6:4) plus 0.5% Br ₂ by vol. | Sudan Black B | a. diagnostic b. bromination on layers | 180 |
| 10 | Triglycerides | Kieselguhr G impregnated with tetradecane | a. acetone-CH ₃ CN (8:2) 80% sat. with tetradecane b. propanoic acid-CH ₃ CN (6:4) 80% sat. with tetradecane plus 0.5% Br ₂ by vol. | a. I ₂ b. phosphomolybdic acid | a. diagnostic b. bromination on layer | 186 |
| | | Kieselguhr G impregnated with paraffin (b.p. 240-250°) or Silicone oil | c. acetone-CH ₃ CN (8:2) d. MeOH-CH ₃ CN (5:4) e. MeOH-CH ₃ CN-CH ₃ CH ₂ CN (5:4:1.5) f. solution e saturated with silicone oil | c. Rhodamine B d. I ₂ e. phosphomolybdic acid | diagnostic | 183 |
| 11 | Glycerides of milk fat (lipolyzed) | Silica Gel G | pet. ether-EtOH-HOAc (90:10:1) | | preparative separation prior to assay by gas chromatography | 178 |
| 12 | Glycerides of linseed oil | plaster-of-Paris-bound silicic acid | gradient elution from pet. ether to pet. ether-EtOEt (1:1) | conc. H ₂ SO ₄ | a. diagnostic b. quantitative c. used to find optimum reaction conditions | 335 |
| 13 | C ¹⁴ and tritium assay of triphosphoinositidyl fatty acids | Silica Gel G | pet. ether-EtOEt-HOAc (90:10:1) | a. I ₂ b. 2',7'-dichlorofluorescein | a. quantitative b. preparative | 364 |
| 14 | Alkoxylglycerides | | | | diagnostic | 212 |
| 15 | Acetoglycerides | silicic acid | "Skellysolve" F-EtOEt (92:8) | I ₂ | diagnostic | 130 |
| 16 | Glycerides of the Swiss Pharmacopoeia | Kieselguhr G impregnated with paraffin HOAc | | a. I ₂ and benzidine b. Rhodamine B and KOH | diagnostic | 6 |
| 17 | Glycerides from plasma | Silica Gel G | pet. ether-methyl ethyl ketone-HOAc (8:15:1) | a. 50% H ₂ SO ₄ b. osmium tetroxide c. Rhodamine 6 G | diagnostic | 459 |

TABLE II.15. LIPIDS—GLYCOLIPIDS

| Compound | Adsorbent | Developer | Visualization | Comments | | Ref. |
|------------------|--------------|---|---|---|---|------|
| | | | | a. diphenylamine | a. diagnostic b. stepwise tech- nique | |
| 1 Glycolipids | Silica Gel G | a. PrOH-NH ₄ OH (12% NH ₃)(4:1) b. 1,2-dichloroethane-MeOH (49:1) c. CHCl ₃ -96% HOAc (95:5) all consecutively | diphenylamine | a. diagnostic b. stepwise tech- nique | 174 | |
| 2 Glycolipids | Silica Gel G | CHCl ₃ -MeOH-H ₂ O (60:35:8) (65:25:4) | a. Rhodamine B b. Dragendorff's reag. c. Bial's reagent d. diphenylamine | diagnostic | 427 432 | |

TABLE 11.16. LIPIDS—PHOSPHOLIPIDS AND SULFOLIPIDS

| | Compound | Adsorbent | Developer | Visualization | Comments | Ref. |
|----|--|--|---|---|--------------|------------|
| 1 | Cephalins and lecithins | Silica Gel G | $\text{CHCl}_3\text{-MeOH-H}_2\text{O}$ (11:5:1) | phosphomolybdc acid-SnCl ₂ on layer "smoked" with dichlorodimethylsilane | diagnostic | 185 |
| 2 | Sphingosines, etc. | silicic acid on walls of test tubes | a. CHCl_3 b. $\text{NH}_3\text{-MeOH-CHCl}_3$ (20:80) (30:70) (NH_3) ₂ MeOH is $\text{MeOH-H}_2\text{O-NH}_3\text{OH}$ (28% NH_3) (93:5:2) | I_2 | diagnostic | 219 |
| 3 | Lecithin and colaminecephalin | Silica Gel G | $\text{CHCl}_3\text{-MeOH-H}_2\text{O}$ (65:25:4) | a. Rhodamine B b. Dragendorff's reag. c. ninhydrin | quantitative | 430 |
| 4 | Misc. phospholipids | Silica Gel G | $\text{CHCl}_3\text{-MeOH-H}_2\text{O}$ (60:35:8) (65:25:4) | a. Rhodamine B b. Dragendorff's reag. c. ammonium molybdate- HClO_4 d. ninhydrin | diagnostic | 427 432 |
| 5 | Serum phospholipids | plaster-of-Paris-bound silicic acid | $\text{CHCl}_3\text{-MeOH-H}_2\text{O}$ (80:25:3) | $2',7'$ -dichlorofluorescein | diagnostic | 423 |
| 6 | Lecithins, cephalins and sphingomyelins | Silica Gel G with 10% $(\text{NH}_4)_2\text{SO}_4$ | $\text{CHCl}_3\text{-H}_2\text{SO}_4\text{-MeOH}$ (80:20) (H_2SO_4) MeOH is 0.1 N aqueous $\text{H}_2\text{SO}_4\text{-MeOH}$ (5:95) | a. I_2 b. $2',7'$ -dichlorofluorescein c. $\text{K}_3\text{Cr}_2\text{O}_7\text{-H}_2\text{SO}_4$ | diagnostic | 234 |
| 7 | Cerebrin, kerasin and their sulfuric acid esters | Silica Gel G | a. 1,2-dichloroethane b. $\text{CHCl}_3\text{-HOAc}$ (96:4) c. $\text{PrOH-NH}_3\text{OH}$ (12.5% NH_3) (8:2) | bromthymol blue | diagnostic | 173 |
| 8 | Beef brain gangliosides | Silica Gel G | $\text{PrOH-H}_2\text{O}$ (7:3) | a. bromthymol blue b. Ehrlich reagent | preparative | 208 |
| 9 | Cerebral gangliosides | Silica Gel G | $\text{BuOH-pyridine-H}_2\text{O}$ (3:2:1) | Bial's reagent | diagnostic | 197 |
| 10 | Beef brain gangliosides | Silica Gel G | $\text{PrOH-1}N\text{ NH}_3\text{OH-H}_2\text{O}$ (6:2:1) | $\text{PrOH-1}N\text{ NH}_3\text{OH-H}_2\text{O}$ (6:2:1) | diagnostic | 440 |

TABLE 11.15. LIPIDS—GLYCOLIPIDS

| | Compound | Adsorbent | Developer | Visualization | Comments | Ref. | |
|---|-------------|--------------|---|---|------------|------------|-----|
| 1 | Glycolipids | Silica Gel G | a. PrOH-NH ₄ OH (12% NH ₃)(4:1) b. 1,2-dichloroethane-MeOH (49:1) c. CHCl ₃ -96% HOAc (95:5) all consecutively | diphenylamine | | | 174 |
| | | | | a. diagnostic b. stepwise tech- nique | | | |
| | | | | | | | |
| 2 | Glycolipids | Silica Gel G | CHCl ₃ -MeOH-H ₂ O (60:35:8) (65:25:4) | a. Rhodamine B b. Dragendorff's reag. c. Bial's reagent d. diphenylamine | diagnostic | 427 432 | |

TABLE 11.16. LIPIDS—PHOSPHOLIPIDS AND SULFO-LIPIDS

| | Compound | Adsorbent | Developer | Visualization | Comments | Ref. |
|----|--|---|---|---|--------------|------------|
| 1 | Cephalins and lecithins | Silica Gel G | CHCl ₃ -MeOH-H ₂ O (11:5:1) | phosphomolybdic acid-SnCl ₂ on layer, "smoked" with dichlorodimethylsilane | diagnostic | 185 |
| 2 | Sphingosines, etc. | silicic acid on walls of test tubes | a. CHCl ₃ b. NH ₃ /MeOH-CHCl ₃ (20:80) (30:70) (NH ₃ /MeOH is MeOH-H ₂ O-NH ₃ OH (38% NH ₃) (33:5:2)) | I ₂ | diagnostic | 219 |
| 3 | Lecithin and colaminecephalin | Silica Gel G | CHCl ₃ -MeOH-H ₂ O (65:25:4) | a. Rhodamine B b. Dragendorff's reag. c. ninhydrin | quantitative | 430 |
| 4 | Misc. phospholipids | Silica Gel G | CHCl ₃ -MeOH-H ₂ O (60:35:8) (65:25:4) | a. Rhodamine B b. Dragendorff's reag. c. ammonium molybdate-HClO ₄ d. ninhydrin | diagnostic | 427 432 |
| 5 | Serum phospholipids | plaster-of-Paris-bound silicic acid | CHCl ₃ -MeOH-H ₂ O (80:25:3) | 2',7'-dichlorofluorescein | diagnostic | 423 |
| 6 | Lecithins, cephalins and sphingomyelins | Silica Gel G with 10% (NH ₄) ₂ SO ₄ | CHCl ₃ -H ₂ SO ₄ /MeOH (80:20) (H ₂ SO ₄ /MeOH is 0.1 N aqueous H ₂ SO ₄ -MeOH (5:95)) | a. I ₂ b. 2',7'-dichlorofluorescein c. K ₂ Cr ₂ O ₇ -H ₂ SO ₄ | diagnostic | 234 |
| 7 | Cerebrin, kerasin and their sulfuric acid esters | Silica Gel G | a. 1,2-dichloroethane b. CHCl ₃ -HOAc (96:4) c. P ₂ O ₅ -NH ₄ OH (12.5% NH ₃) (8:2) | bromthymol blue | diagnostic | 173 |
| 8 | Beef brain gangliosides | Silica Gel G | PrOH-H ₂ O (7:3) | a. bromthymol blue b. Ehrlich reagent | preparative | 208 |
| 9 | Cerebral gangliosides | Silica Gel G | BuOH-pyridine-H ₂ O (3:2:1) | Bial's reagent | diagnostic | 197 |
| 10 | Beef brain gangliosides | Silica Gel G | PrOH-1 <i>N</i> NH ₄ OH-H ₂ O (6:2:1) | | diagnostic | 440 |

TABLE II.17. NUCLEOTIDES

| | Compound | Adsorbent | Developer | Visualization | Comments | Ref. |
|---|--|---|---|---------------|---|------------|
| 1 | Misc. nucleotides | collodion-bound "ECT-EOLA" cellulose | a. 0.15 M NaCl b. 0.01 N HCl | U.V. | diagnostic | 316 317 |
| 2 | Misc. nucleotides | Cellulose Powder MN 300 G | a. H ₂ O b. sat. aqueous (NH ₄) ₂ SO ₄ -1 M NaOAc- 2-PrOH (90:18:2) c. <i>t</i> -amyl alcohol-formic acid-H ₂ O (3:2:1) d. BuOH-acetone-HOAc-NH ₄ OH (5% NH ₃)-H ₂ O (3:5:2:5:1:5:1:1) | U.V. | a. diagnostic b. comparison with paper chromatog. | 319 |
| 3 | Misc. nucleotides | a. DEAE-cellulose b. cellulose c. ECTEOLA-cellulose | dilute aqueous HCl 0.02 to 0.04 N sat. aqueous (NH ₄) ₂ SO ₄ -1 M NaOAc-2- PrOH (80:18:2) 0.06 N HCl | U.V. | a. diagnostic b. preparative c. review | 320 318 |
| 4 | Misc. nucleotides | Cellulose Powder MN 300 | a. H ₂ O for nucleobases b. BuOH-acetone-HOAc-NH ₄ OH (5% NH ₃)-H ₂ O (4:5:1.5:1:1:2) | U.V. | diagnostic | 321 |
| 5 | Nucleotides, AMP, ADP and ATP | "DEAE-Sephadex" A-25 | gradient elution starting with 1.0 N formic acid and adding 10 N formic acid which is 2 M in ammonium formate | fluorescein | diagnostic | 82 |
| 6 | Amino acid esters as models for amino- acrylribonucleic acid | see "Proteins, etc." No. 18 | | | | 456 |

TABLE 11.18. PHENOLS, ALCOHOLS AND THEIR DERIVATIVES

| | Compound | Adsorbent | Developer | Visualization | Comments | Ref. |
|----|---------------------------------------|---|--|---|---------------------------------|------|
| 1 | Acetylene alcohols and glycols | | | | | 4 |
| 2 | Chimyl, batyl and se-lachyl alcohols | see "Lipids-Glycerides," No. 14 | | | | 212 |
| 3 | Long-chain aliphatic alcohols | Silica Gel G | a. pet. ether-EtOEt-HOAc (90:10:1) b. pet. ether-EtOEt (20:80) (10:90)(70:30) | 50% H ₂ SO ₄ | diagnostic | 395 |
| 4 | Cyclohexanol derivatives | Silica Gel G | a. benzene b. benzene-EtOEt (80:20) | H ₂ O | a. diagnostic b. preparative | 128 |
| 5 | Cardanol and its methylol derivatives | plaster-of-Paris-bound silica gel | EtOAc-toluene (60:40) | diazotized sulfanilic acid | followed course of reaction | 11 |
| 6 | Phenolic acids and esters | see "Carboxylic Acids, etc., No. 9 | | | | 69 |
| 7 | Phenols | a. starch-bound silicic acid b. starch-bound silicic acid kieselguhr (1:1) | a. xylene b. xylene-CHCl ₃ (3:1)(1:1)(1:3) c. CHCl ₃ | diazotized sulfanilic acid followed by KOH | diagnostic | 426 |
| 8 | Pyrocatechol and enge-nol derivatives | Silica Gel G, acidic and basic | benzene | SB ₂ Cl ₆ in CCl ₄ | diagnostic | 369 |
| 9 | Phenols and phenolic acids | starch-bound silicic acid with phosphors | a. "Skellysolve" B-EtOEt (3:7) b. "Skellysolve" B-EtOAc (1:3) c. "Skellysolve" B-acetone (3:1) | U.V. | followed course of reaction | 225 |
| 10 | Phloroglucinol derivatives | see "Miscellaneous," No. 15 | | | | 380 |
| 11 | Phenolic aldehydes | see "Aldehydes, etc., No. 9 | | | | 396 |

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TABLE II.17. NUCLEOTIDES

| | Compound | Adsorbent | Developer | Visualization | Comments | Ref. |
|---|---|---|---|---------------|---|----------------|
| 1 | Misc. nucleotides | collodion-bound "ECT-EOLA" cellulose | a. 0.15 M NaCl b. 0.01 N HCl | U.V. | diagnostic | 316 317 |
| 2 | Misc. nucleotides | Cellulose Powder MN 300 G | a. H ₂ O b. sat. aqueous (NH ₄) ₂ SO ₄ -1 M NaOAc- 2-PrOH (80:18:2) c. <i>t</i> -amyl alcohol-formic acid-H ₂ O (3:2:1) d. BuOH-acetone-HOAc-NH ₄ OH (5% NH ₃)-H ₂ O (3:5:2.5:1.5:1.5:1) | U.V. | a. diagnostic b. comparison with paper chromatog. | 319 |
| 3 | Misc. nucleotides | a. DEAE-cellulose b. cellulose c. ECTEOLA-cellulose | dilute aqueous HCl 0.02 to 0.04 N sat. aqueous (NH ₄) ₂ SO ₄ -1 M NaOAc-2- PrOH (80:18:2) 0.06 N HCl | U.V. | a. diagnostic b. preparative c. review | 320 318 |
| 4 | Misc. nucleotides | Cellulose Powder MN 300 | a. H ₂ O for nucleobases b. BuOH-acetone-HOAc-NH ₄ OH (5% NH ₃)-H ₂ O (4:5:1.5:1:1:2) | U.V. | diagnostic | 321 |
| 5 | Nucleotides, AMP, ADP and ATP | "DEAE-Sephadex" A-25 | gradient elution starting with 1.0 N formic acid and adding 10 N formic acid which is 2 M in ammonium formate | fluorescein | diagnostic | 82 |
| 6 | Amino acid esters as models for amino- acylribonucleic acid | see "Proteins, etc., No. 18 | | | | 456 |

TABLE 11.18. PHENOLS, ALCOHOLS AND THEIR DERIVATIVES

| | Compound | Absorbent | Developer | Visualization | Comments | Ref. |
|----|---------------------------------------|---|--|--|---------------------------------|------|
| 1 | Acetylene alcohols and glycols | | | | | 4 |
| 2 | Chimyl, bayl and se-lachyl alcohols | see "Lipids-Glycerides," No. 14 | | | | 212 |
| 3 | Long-chain aliphatic alcohols | Silica Gel G | a. pet. ether-EtOEt-HOAc (90:10:1) b. pet. ether-EtOEt (20:80) (10:90)(70:30) | 50% H ₂ SO ₄ | diagnostic | 395 |
| 4 | Cyclohexanol derivatives | Silica Gel G | a. benzene b. benzene-EtOEt (80:20) | H ₂ O | a. diagnostic b. preparative | 128 |
| 5 | Cardanol and its methylol derivatives | Plaster-of-Paris-bound silica gel | EtOAc-toluene (60:40) | diazotized sulfanilic acid | followed course of reaction | 11 |
| 6 | Phenolic acids and esters etc., No. 9 | see "Carboxylic Acids, etc., No. 9 | | | | 69 |
| 7 | Phenols | a. starch-bound silicic acid b. starch-bound silicic acid kieselguhr (1:1) | a. xylene b. xylene-CHCl ₃ (3:1)(1:1)(1:1) c. CHCl ₃ | diazotized sulfanilic acid followed by KOH | diagnostic | 426 |
| 8 | Pyrocatechol and eugenol derivatives | Silica Gel G, acidic and basic | | SbCl ₃ in CCl ₄ | diagnostic | 369 |
| 9 | Phenols and phenolic acids | starch-bound silicic acid with phosphors | a. "Skellysolve" B-EtOEt (3:1:7) b. "Skellysolve" B-EtOAc (1:3) c. "Skellysolve" B-acetone (3:1) | U.V. | followed course of reaction | 225 |
| 10 | Phloroglucinol derivatives | see "Miscellaneous," No. 15 | | | | 380 |
| 11 | Phenolic aldehydes | see "Aldehydes, etc., No. 9 | | | | 396 |

Thin-Layer Chromatography

TABLE 11.17. NUCLEOTIDES

| | Compound | Adsorbent | Developer | Visualization | Comments | Ref. |
|---|--|---|---|---------------|---|----------------|
| 1 | Misc. nucleotides | collodion-bound "ECT-EOLA" cellulose | a. 0.15 M NaCl b. 0.01 N HCl | U.V. | diagnostic | 316 317 |
| 2 | Misc. nucleotides | Cellulose Powder MN 300 G | a. H ₂ O b. sat. aqueous (NH ₄) ₂ SO ₄ -1 M NaOAc- 2-PrOH (80:18:2) c. t-amyl alcohol-formic acid-H ₂ O (3:2:1) d. BuOH-acetone-HOAc-NH ₄ OH (5% NH ₃)-H ₂ O (3.5:2.5:1.5:1.5:1) | U.V. | a. diagnostic b. comparison with paper chromatog. | 319 |
| 3 | Misc. nucleotides | a. DEAE-cellulose b. cellulose c. EGTEOLA-cellulose | dilute aqueous HCl 0.02 to 0.04 N sat. aqueous (NH ₄) ₂ SO ₄ -1 M NaOAc-2- PrOH (80:18:2) 0.06 N HCl | U.V. | a. diagnostic b. preparative c. review | 320 318 |
| 4 | Misc. nucleotides | Cellulose Powder MN 300 | a. H ₂ O for nucleobases b. BuOH-acetone-HOAc-NH ₄ OH (5% NH ₃)-H ₂ O (4.5:1.5:1:1:2) | U.V. | diagnostic | 321 |
| 5 | Nucleotides, AMP, ADP and ATP | "DEAE-Sephadex" A-25 | gradient elution starting with 1.0 N formic acid and adding 10 N formic acid which is 2 M in ammonium formate | fluorescein | diagnostic | 82 |
| 6 | Amino acid esters as models for amino- acyribonucleic acid | see "Proteins, etc.," No. 18 | | | | 456 |

TABLE 11.18. PHENOLS, ALCOHOLS AND THEIR DERIVATIVES

| | Compound | Adsorbent | Developer | Visualization | Comments | Ref. |
|----|---------------------------------------|---|--|--|---------------------------------|------|
| 1 | Acetylene alcohols and glycols | | | | | 4 |
| 2 | Chimyl, baylyl and se-lachyl alcohols | see "Lipids-Glycerides," No. 14 | | | | 212 |
| 3 | Long-chain aliphatic alcohols | Silica Gel G | a. pet. ether-EtOEt-HOAc (90:10:1) b. pet. ether-EtOEt (20:80) (10:90)(70:30) | 50% H ₂ SO ₄ | diagnostic | 395 |
| 4 | Cyclohexanol derivatives | Silica Gel G | a. benzene b. benzene-EtOEt (80:20) | H ₂ O | a. diagnostic b. preparative | 128 |
| 5 | Cardanol and its methylol derivatives | plaster-of-Paris-bound silica gel | EtOAc-toluene (60:40) | diazotized sulfanilic acid | followed course of reaction | 11 |
| 6 | Phenolic acids and esters | see "Carboxylic Acids, etc.," No. 9 | | | | 69 |
| 7 | Phenols | a. starch-bound silicic acid b. starch-bound silicic acid kieselguhr (1:1) | a. xylene b. xylene-CHCl ₃ (3:1)(1:1)(1:3) c. CHCl ₃ | diazotized sulfanilic acid followed by KOH | diagnostic | 426 |
| 8 | Pyrocatechol and eugenol derivatives | Silica Gel G, acidic and basic | benzene | SbCl ₃ in CCl ₄ | diagnostic | 369 |
| 9 | Phenols and phenolic acids | starch-bound silicic acid with phosphors | a. "Skellysolve" B-EtOEt (3:7) b. "Skellysolve" B-EtOAc (1:3) c. "Skellysolve" B-acetone (3:1) | U.V. | followed course of reaction | 225 |
| 10 | Phloroglucinol derivatives | see "Miscellaneous," No. 15 | | | | 380 |
| 11 | Phenolic aldehydes | sec "Aldehydes, etc.," No. 9 | | | | 396 |

TABLE 11.18—*Cont.*

| Compound | Absorbent | Developer | Visualization | Comments | Ref. |
|---|---|--|---|---|------------|
| 12 Phenolic aldehydes, ketones and acids | Silica Gel G | a. benzene-dioxane-HOAc (90:25:4) b. benzene-MeOH-HOAc (45:8:4) c. benzene-MeOH (95:5) | a. diazotized benzidine b. alkaline KMnO ₄ c. diazotized sulfanilic acid d. Rhodamine B | a. diagnostic b. quantitative | 288 290 |
| 13 Phenols and glycols | a. unbound aluminum oxide with HOAc | a. benzene | paper chromatography reagents | diagnostic | 144 |
| Nitro and amino phenols | b. unbound aluminum oxide | b. benzene-EtOH mixtures c. CHCl ₃ d. EtOEt | | | |
| 14 Phenols | Plaster-of-Paris-bound silica gel-oxalic acid | a. hexane-EtOAc (4:1)(3:2) b. benzene-ethyl ether (4:1) c. benzene | | diagnostic | 113 |
| 15 3,5-Dinitrobenzoates of alcohols and phenols | Silica Gel G | a. benzene-pet. ether (1:1) b. hexane-EtOAc (85:15)(75:25) c. toluene-EtOAc (90:10) | colored compounds | does not separate closely related compounds | 85 |
| 16 3,5-Dinitrobenzoates of alcohols | starch-bound silica gel-bentonite (4:1) with fluorescent material | hexane | U.V. | diagnostic | 209 |
| 17 3,5-Dinitrobenzoates of butyl and isoctyl alcohols | silica gel | | colored compounds | | 220 |
| 18 Phenols in pharmaceuticals | unbound acidic aluminum oxide | a. ether b. benzene-EtOH (95:5)(90:10) | a. KMnO ₄ -Fe(No ₃) ₃ b. KMnO ₄ | diagnostic | 340 |
| 19 Phenolic carboxylic acids | Kieselguhr G with Na ₂ WO ₄ , NaMoO ₄ or borax | a. organic layer of BuOH-HOAc-H ₂ O (4:1:5) b. butyl ether (sat. with H ₂ O)-HOAc (10:1) c. EtOAc-2-P-OH-H ₂ O (65:24:11) | Folin Denis Reagent | | 136 |

| | | | | | |
|----|----------|-----------------------------|--|--|--------------|
| 20 | Xylenols | Silica Gel G | a. benzene b. benzene-MeOH (95:5) | a. diazotized benzidine b. Echt blue salt | 301 |
| 21 | Alcohols | | | | 28 |
| 22 | Phenols | Silica Gel G and boric acid | EtOH-H ₂ O (90:30) containing 4% boric acid and 2% NaOAc as electrolyte | Diazotized benzidine | 291 |
| 23 | Phenols | ϵ -polycaprolactam | a. hexane b. cyclohexane c. benzene d. CHCl ₃ e. EtOAc | diazotized sulfanilic acid | 219a 436a |

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TABLE 11.19. PROTEINS, AMINO ACIDS AND THEIR DERIVATIVES

| Compound | Absorbent | Developer | Visualization | Comments | Ref. |
|--------------------------------------|---|--|-----------------------|---|-----------|
| 1 Amino acids | Silica Gel G | a. EtOH (96%)-H ₂ O (63:37) b. PrOH-H ₂ O (64:36) c. BuOH-HOAc-H ₂ O (60:20:20) d. phenol-H ₂ O (75:25) e. PrOH-NH ₄ OH (34% NH ₃) (67:33) f. EtOH (96%)-NH ₄ OH (34% NH ₃) (77:23) | modified ninhydrin | a. diagnostic b. two dimensional | 44 277 |
| 2 Amino acids | unbound aluminum oxide | a. H ₂ O b. pyridine-H ₂ O (1:1)(80:54) c. BuOH-EtOH-H ₂ O (60:40:40) | ninhydrin | a. diagnostic b. continuous elution c. spotted sample with NaOH | 267 |
| 3 Amino acids | buffered Silica Gel G (buffered with equal portions of 0.2 M KH ₂ PO ₄ and 0.2 M Na ₂ HPO ₄) | a. EtOH-H ₂ O (70:30) b. EtOH-NH ₄ OH (25% NH ₃) (4:1) c. EtOH-NH ₄ OH-H ₂ O (7:1:2) | paper chrom. reagents | diagnostic | 271 |
| 4 Amino acids | Silica Gel G | a. CH ₃ Cl-MeOH-NH ₄ OH (17% NH ₃) (2:2:1) in first direction b. phenol-H ₂ O (75:25 g/g) in second dimension c. methyl ethyl ketone-pyridine-H ₂ O-HOAc (70:15:15:2) for continuous | ninhydrin | a. diagnostic b. continuous development c. two dimensional | 99 |
| 5 Amino acids | Cellulose MN 300 Powder | a. upper phase of BuOH-HOAc-H ₂ O (4:1:5) b. pyridine-methyl ethyl ketone-H ₂ O (15:70:15) c. MeOH-H ₂ O-pyridine (80:20:4) d. six others | ninhydrin | diagnostic | 430 |
| 6 Amino acids | Silica Gel G | a. PrOH-H ₂ O (1:1) in first direction b. phenol-H ₂ O (10:4) in second direction | ninhydrin | a. diagnostic b. two dimensional | 279 |
| 7 Amino acids and similar substances | a. Silica Gel G b. Kieselguhr G c. Aluminum Oxide G | a. 440V, 19.6 m. amp. in 0.1 M sodium citrate buffer, pH 3.8 b. 460V, 12.6 m. amp. in 2 N HOAc-0.6 N formic acid (1:1) at pH 2 c. chromatography using BuOH-HOAc-H ₂ O (3:1:1) | ninhydrin | a. diagnostic b. electrophoresis c. combined electrophoresis and chromatog. | 136 |

| | | | | | | |
|----|--|--|---|--|---|-----|
| 8 | N-(2,4-Dinitrophenyl)amino acids | a. Silica Gel G b. Silica Gel G with phosphor | a. toluene-pyridine-ethylenechlorohydrin-0.8 NH ₄ OH (100:30:60:60), upper phase, in first direction. b. CHCl ₃ -benzyl alcohol-HOAc (70:30:3) in second direction c. Sol. a in one direction and CHCl ₃ -MeOH-HOAc (95:5:1) in the second direction as continuous chrom. d. Sol. a in one direction and benzene-pyridine-HOAc (30:20:2) in second direction as continuous chrom. e. CHCl ₃ - <i>t</i> -amyl alcohol-HOAc (70:30:3) | <i>N</i> a. colored compounds b. transmitted U.V. c. U.V. | a. Layers are all equilibrated with lower phase of sol. a before use b. continuous development c. two dimensional | 45 |
| 9 | Cit-auged N-(2,4-dinitrophenyl)-amino acids | Silica Gel G | a. toluene-pyridine-ethylenechlorohydrin-0.8 NH ₄ OH (100:30:60:60), upper phase in first direction b. CHCl ₃ -benzylalcohol-glac. HOAc (70:30:3) in second direction | radiographic Cl ₂ -toluidine | a. two dimensional b. layer equilibrated with lower phase of sol. a before use | 89 |
| 10 | 2-Phenyl-2-thiobutydantsins of amino acids | Silica Gel G | a. CHCl ₃ b. CHCl ₃ -MeOH (9:1) c. CHCl ₃ -formic acid (100:5) d. CHCl ₃ -MeOH-formic acid (70:30:2) e. Methyl ethyl ketone-pyridine-H ₂ O-conc. HOAc (70:15:15:2) | Cl ₂ -toluidine | diagnostic | 46 |
| 11 | Carbobenzoxy derivatives of amino acids and small peptides | Silica Gel G | a. BuOH-acetone-glac. HOAc-NH ₄ OH (7% NH ₃)-H ₂ O (4:5:1.5:1:1:2) b. BuOH-glac. HOAc-NH ₄ OH (7% NH ₃) (5:5:3:1:5) c. BuOH-HOAc-NH ₄ OH (7% NH ₃)-H ₂ O (6:1:1:2) d. BuOH-glac. HOAc-H ₂ O-pyridine (15:3:12:10) e. in case of two-phase systems, both were used, but layer dipped only in upper phase | a. ninhydrin b. K ₂ Cr ₂ O ₇ in conc. H ₂ SO ₄ | diagnostic | 95 |
| 12 | Amino acid and peptide derivatives | Silica Gel G | CHCl ₃ -MeOH (9:1) | Folin reagent | a. diagnostic b. monitored a synthesis | 163 |
| 13 | Polypeptides (up to five units) and derivatives | Silica Gel G | a. CHCl ₃ -acetone (9:1)(8:2) b. cyclohexane-EtOAc (1:1) c. CHCl ₃ -MeOH (9:1) | morin | diagnostic | 341 |

TABLE II.19—*Cont.*

| Compound | Absorbent | Developer | Visualization | Comments | Ref. |
|---|----------------------------------|--|---|---|------|
| 14 Polypeptides (up to ten units) and derivatives | Silica Gel G | a. BuOH sat. with 0.1% NH ₄ OH b. other systems used in paper chrom. | ninhydrin | a. diagnostic b. monitored a synthesis | 424 |
| 15 3-phenyl-2-thiohydantoin derivatives of di- and tripeptides | Silica Gel G | neptane-pyridine-EtOAc (5:3:2) | starch-iodine azide | diagnostic | 54 |
| 16 Proteins as such | polyamide-bound hydroxyl-apatite | potassium phosphate buffers, pH 6.5 of various concentrations | ninhydrin | a. diagnostic b. gradient elution | 153 |
| 17 Proteins and poly-peptides | Sephadex G 25 (moist) | a. 0.05 M NH ₄ OH b. H ₂ O | a. Pauli's reagent | a. diagnostic b. determine mol. wt. | 82 |
| Enzymes | "DEAE-Sephadex" A 25 | c. gradient elution involving addition of 0.02 M phosphate buffer pH 7.2 (which is also 1 M in NaCl) to the pure buffer | b. DPNH-pyruvic acid | c. gradient elution d. preparative | |
| 18 Carbobenzoxyaminoacyl derivatives of cyclic alcohols | Silica Gel G | CHCl ₃ -acetone (85:15) | Cl ₂ followed by KI and starch | a. monitored course of reaction b. monitored column chrom. | 456 |
| 19 Metabolic products of tryptophane | Silica Gel G | a. CHCl ₃ -MeOH-HOAc (75:20:5) b. methyl acetate-2-PrOH-NH ₄ OH (25% NH ₃) (45:35:20) | Ehrlich reagent | a. diagnostic b. urine analysis | 87 |

TABLE 11.20. STEROIDS

| | Compound | Adsorbent | Developer | Visualization | Comments | Ref. |
|---|--------------------------------------|--|--|--|---|------|
| 1 | Seventeen misc. steroids | Silica Gel G | a. benzene b. benzene-EtOAc (9:1)(2:1) c. cyclohexane-EtOAc (9:1)(19:1) d. 1,2-dichloroethane e. seven more | SbCl ₃ in CHCl ₃ | diagnostic | 416 |
| 2 | Seventy-nine steroids and sapogenins | unbound aluminum oxide, sometimes impregnated with morin | a. hexane-benzene mixtures b. benzene-EtOEt mixtures c. EtOEt-EtOH mixtures | a. conc. H ₂ SO ₄ b. U.V. c. morin spray | a. diagnostic b. preparative | 52 |
| 3 | Twenty-two misc. steroids | a. starch-bound silic acid b. Silica Gel G | EtOAc-cyclohexane mixtures | a. SbCl ₃ in CHCl ₃ b. 2,4-DNPH c. phosphomolybdic acid | diagnostic | 13 |
| 4 | Twenty-seven steroid estrogens | Silica Gel G | a. cyclohexane-EtOAc-EtOH (45:45:10) b. EtOAc-cyclohexane-EtOH (80:15:5) c. EtOAc-cyclohexane (50:50) d. CHCl ₃ -EtOH (90:10) e. EtOAc-cyclohexane-EtOH-HOAc (72:15:5:4:5:10) f. BuOH sat. with H ₂ O | a. Folin-Ciocalteau reag. b. Boure reagent c. 2% H ₂ SO ₄ in H ₂ O-EtOH (1:1) | a. diagnostic b. classification system | 221 |
| 5 | Forty-nine misc. steroids | unbound aluminum oxide | a. benzene-pet. ether mixtures b. benzene-EtOH mixtures | a. SbCl ₃ in CHCl ₃ b. SbCl ₃ in CHCl ₃ with 10% SOCl ₂ | diagnostic | 145 |
| 6 | Twenty-seven misc. steroids | a. unbound aluminum oxide | a. pet. ether-benzene mixtures b. benzene c. benzene-EtOH mixtures | reagents used in paper chromatography | diagnostic | 144 |
| | Steroid acids | b. unbound aluminum oxide-HOAc | d. benzene-EtOH (90:10) | | | |

Thin-Layer Chromatography

TABLE 11.20—*Cont.*

| Compound | Adsorbent | Developer | Visualization | Comments | Ref. |
|---|---|---|--|----------------------------------|------|
| 7 Steroids from fermentation reactions | Silica Gel G | CHCl ₃ -acetone mixtures | a. vanillin in H ₃ PO ₄ b. anisaldehyde in H ₃ SO ₄ c. SbCl ₃ in CHCl ₃ d. H ₃ PO ₄ e. 2,3,5-triphenyltetrazolium chloride f. HClO ₄ | diagnostic | 247 |
| 8 Thirteen misc. steroids | a. Silica Gel G with phosphor b. Aluminum Oxide G with phosphor | a. CHCl ₃ -EtOAc (3:2)(3:1) b. benzene-EtOAc (1:1)(9:1) c. hexane-EtOAc (1:1) | I ₂ vapor | a. quantitative b. diagnostic | 244 |
| 9 Fifty-five misc. steroids | a. starch-bound silica gel b. starch-bound silica gel-NaOH | a. hexane-EtOAc (4:1)(1:1) b. EtOAc c. benzene-2-PrOH (4:1) | a. phosphomolybdic acid b. many others | diagnostic | 363 |
| 10 Nineteen misc. steroids | plaster-of-Paris-bound “Celite” impregnated, by spraying, with a. formamide b. propylene glycol c. paraffin oil | a. hexane b. hexane-benzene (1:1) c. benzene d. CHCl ₃ e. ligroin f. MeOH-H ₂ O (95:5) | a. H ₃ PO ₄ b. tetrazolium blue c. isonicotinic acid hydrazide | a. diagnostic b. distribution | 414 |
| 11 Fifteen sterols and sterol derivatives | a. Silica Gel G b. Silica Gel G Kieselgur G (1:1) | a. cyclohexane-EtOAc-H ₂ O (600:400:1) (1560:440:1) b. isoctane-CCl ₄ (19:1) c. cyclohexane-heptane (1:1) | 50% H ₂ SO ₄ | diagnostic | 27 |

| 295 | | | | | |
|-----|-----------------------------|---|---|---|--|
| 12 | Sterols and sterol acetates | a. Kieselguhr G b. Kieselguhr G impregnated with undecane | a. cyclohexane-EtOAc (9:5:0.5) b. HOAc-H ₂ O (92:8)(90:10) c. HOAc-CH ₂ CN (1:3) | phosphomolybdic acid | diagnostic |
| 26 | | a. Silica Gel G b. Kieselguhr G c. a and b (1:1) d. all equilibrated with water by placing moist filter paper in the chamber | a. CH ₂ Cl ₂ -MeOH-formamide (93:6:1) b. toluene-Et ₂ O-formic acid (57:40:3) c. cyclohexane-acetone (1:1) d. cyclohexane-EtOAc-H ₂ O (600:400:1) e. CH ₂ Cl ₂ -MeOH-H ₂ O (485:15:1) f. three others | a. diagnostic b. distribution chrom. | |
| 405 | | Sllica Gel G | a. EtOAc b. butyl acetate | chlorosulfonic acid-HOAc (1:2) | diagnostic |
| 67 | | Sllica Gel G | a. benzene b. CHCl ₃ c. CHCl ₃ -MeOH (10:1)(97:3) | SbCl ₃ in CHCl ₃ | diagnostic |
| 68 | | Sllica Gel G | EtOAc-cyclohexane mixtures | SbCl ₃ in CHCl ₃ | diagnostic |
| 125 | | Sllica Gel G | benzene-EtOH (9:1) | SbCl ₅ in CCl ₄ | diagnostic |
| 394 | | Sllica Gel G | benzene-EtOH (9:1) | SbCl ₅ in CHCl ₃ | a. diagnostic b. quantitative |
| 391 | | unbound aluminum oxide | benzene-EtOAc mixtures | 70% H ₃ PO ₄ | a. diagnostic b. quantitative |
| 15 | | plaster-of-Paris-bound silicic acid | pentane-EtOAc mixtures | SbCl ₅ in CCl ₄ | diagnostic |
| 347 | | a. aluminum oxide b. Silica Gel Reidel de Haen | a. benzene-CH ₂ Cl ₂ (2:1) b. cyclohexane-CH ₂ Cl ₂ (1:2) | I ₂ in KI | diagnostic |
| 408 | | Sllica Gel G | a. isopropyl ether b. isopropyl ether-EtOAc (5:2) c. benzene | chlorosulfonic acid-HOAc (1:2) | a. diagnostic b. preparative c. monitored course of reaction |
| | 21 | Misc. derivatives of pregnanol, cholesterol and sitosterol | | | |

Thin-Layer Chromatography

TABLE 11.20—Cont.

| Compound | Adsorbent | Developer | Visualization | Comments | Ref. |
|---|---|---|---|---|------------|
| 22 Pregnadiol | Silica Gel G | CHCl ₃ -acetone (9:1) | <i>ortho</i> -H ₃ P _O ₄ -phosphomolybdc acid | early test for pregnancy | 433 435 |
| 23 Cholesterol and its laurate ester | Silica Gel G | benzene-MeOH mixtures | conc. H ₂ SO ₄ | quantitative | 315 |
| 24 Cholesterol | Silica Gel G | hexane-EtOEt-HOAc (50:50:2) | HClO ₄ | a. quantitative b. microscope slides | 296 |
| 25 Blood cholesterol and cholesterol esters | Silica Gel G | a. benzene b. benzene-EtOAc (9:1) c. 1,2-dichloroethane d. CHCl ₃ e. two others | SbCl ₃ in CHCl ₃ | a. diagnostic b. semiquantitative | 415 |
| 26 Cholesterol esters | Silica Gel G | isopropyl ether-pet. ether (1:99) | SbCl ₃ in CHCl ₃ | a. diagnostic b. quantitative | 460 |
| 27 Cholesterol esters | Silica Gel G | benzene-pet. ether (40:60) | 50% H ₂ SO ₄ | diagnostic | 228 |
| 28 Cholesterol esters | plaster-of-Paris-bound silicic acid impregnated with paraffin | HOAc | a. I ₂ vapor b. SbCl ₃ in CHCl ₃ c. phosphonodibasic acid d. phosphotungstic acid | a. diagnostic b. reversed phase | 249 |
| 29 Bile acids, free and conjugated | Silica Gel G | a. HOAc-C ₂ H ₅ -isopropyl ether-isooamyl acetate-PtOH-benzenc (5:20:30:40: 10:10) for free acids b. propanoic acid-isooamyl acetate-H ₂ O-PtOH (15:20:5:10) for conjugate acids | phosphomolybdc acid | diagnostic | 154 |

| | | | | | | |
|----|---|------------------------------|--|--|---------------------------------------|-----|
| 30 | Cholic acid derivatives | Silica Gel G | a. toluene-HOAc-H ₂ O (5:5:1) upper phase b. BuOH-HOAc-H ₂ O (10:1:1) c. toluene-HOAc-H ₂ O (10:10:1) | a. phosphomolybdic acid b. H ₂ O | a. diagnostic b. quantitative | 115 |
| 31 | Adrenocorticotrophic hormones | Silica Gel G | EtOAc-sylyene-MeOH (90:5:5) | terazolum chloride | diagnostic | 30 |
| 32 | Corticoids from urine | unbound silica gel | CHCl ₃ -EtOH (95:5) | terazolum blue | diagnostic | 1 |
| 33 | Corticosteroids | Silica Gel G (not activated) | CHCl ₃ -MeOH-H ₂ O (485:15:1) (183:12:1)(90:10:1) | 50% H ₂ SO ₄ | diagnostic | 25 |
| 34 | Corticosteroids | plaster of Paris | a. acetone-benzene (1:4) b. EtOH-CHCl ₃ (3:97) | alkaline tetrizolum blue | diagnostic | 243 |
| 35 | Cholestanate derivatives | Silica Gel G | pet. ether-benzene (9:1) | I ₂ | diagnostic | 361 |
| 36 | Cholestanol derivatives | Silica Gel G | a. benzene b. benzene-Et ₂ OEt (70:30) c. pentane-EtOEt-benzene (20:20:60) | H ₂ O | quantitative | 125 |
| 37 | Aglycones of olean- der glycosides | Silica Gel G | EtOAc-CHCl ₃ (9:1) | SbCl ₃ in CHCl ₃ | diagnostic | 126 |
| 38 | Cardinolides | Silica Gel G (not activated) | benzene-EtOH (3:1) sat. with H ₂ O | a. trichloroacetic acid-chloramine-T b. SbCl ₃ -acetic anhydride | a. diagnostic b. preparative | 322 |
| 39 | Steroidal compounds involved in tomatin formation | Silica Gel G | EtOAc-cyclohexane (1:1)(3:7) | a. SbCl ₃ in chloroform b. 15% H ₃ PO ₄ | diagnostic | 337 |
| 40 | Digitoxigenin and derivatives | Silica Gel G | CHCl ₃ -EtOAc (1:1) | trichloroacetic acid-chloramine T | predicted solvents for column chrom. | 339 |
| 41 | Bile acids (free, conjugated) and derivatives | Silica Gel G | a. isomanyl acetate-C ₆ H ₅ -isopropyl ether-benzene-PrOH-H ₂ OAc (4:3:2:1:1:0.5) b. isomanyl acetate-propanoic acid-P ₂ O ₅ -H ₂ O (4:3:2:1) c. cyclohexane-EtOAc (6:4) | phosphomolybdic acid | a. diagnostic b. microscope slides | 90 |
| | | | | | | 152 |

Thin-Layer Chromatography

TABLE 11.20—Cont.

| | Compound | Absorbent | Developer | Visualization | Comments | Ref. |
|----|--|---|---|--|---|------|
| 42 | Steroids from bull testicles | a. plaster-of-Paris-bound silica gel b. EtOAc c. butyl acetate d. several others | a. benzene-EtOAc (8:2) b. EtOAc c. butyl acetate d. several others | conc. H ₂ SO ₄ -U.V. | diagnostic | 272 |
| 43 | Radioactive androstanic acid derivatives | Silica Gel G | cyclohexane-EtOAc (3:2) | radiographic | a. diagnostic b. quantitative c. preparative | 349 |
| 44 | β -Sitossterol from potato beetles | Silica Gel Riedel dc Haen | pet. ether-EtOAc-benzene (85:10:5) | SbCl ₃ in HOAc | diagnostic | 348 |
| 45 | 1,3-Cholestadiene methyl ether | Silica Gel G | benzene-EtOEt (4:1) | I ₂ vapor | monitored column chrom. | 397 |
| 46 | Aldosterones from urine | Silica Gel G | a. cyclohexane-2-PROH (7:3) b. CHCl ₃ glac. HOAc (8:2) | tetrazolium blue | a. two dimensional b. developed in dark c. quantitative | 278 |
| 47 | Saponin from <i>Solanum</i> species | Kieselguhr G | EtOAc-cyclohexane (7:3) | vanillin in H ₃ PO ₄ | diagnostic | 336 |

TABLE 11.21. TERPENOIDS

| | Compound | Adsorbent | Developer | Visualization | Comments | Ref. |
|---|------------------------------------|--|---|--|---|------------|
| 1 | Forty-nine misc. terpenoids | starch-bound silicic acid | hexane | a. fluorescein-Br ₂ b. H ₂ SO ₄ -HNO ₃ | a. diagnostic b. monitored column | 190 |
| 2 | Fourteen misc. terpenoid | starch-bound silicic acid with phosphors | hexane-EtOAc (85:15) | a. fluorescein-Br ₂ b. U.V. c. σ -dianisidine d. conc. H ₂ SO ₄ e. bromoresol green f. H ₂ SO ₄ -HNO ₃ | a. diagnostic b. predicted solvents for column chrom. c. monitored column | 193 251 |
| 3 | Twenty-eight oxygenated terpenoids | starch-bound silicic acid | a. hexane-EtOAc (85:15) (10:30) b. CHCl ₃ (alcohol-free) EtOAc (90:10) (95:5) c. 1-nitropropane-hexane (50:50) d. ethyl carbonate-CHCl ₃ (15:85) e. hexane-isopropyl formate (70:30) f. four others | a. fluorescein-Br ₂ b. σ -dianisidine c. H ₂ SO ₄ -HNO ₃ d. conc. H ₂ SO ₄ | a. diagnostic b. chemical reactions on layers | 252 |
| 4 | Twelve misc. terpenoids | unbound alumina | a. pet. ether-benzene mixtures b. benzene c. benzene-EtOH mixtures | resents used for paper chromatography | diagnostic | 144 |
| 5 | Diterpenoids | silicic acid | hexane-EtOAc (4:1) | KMnO ₄ | a. diagnostic b. monitored column c. review | 213 |
| 6 | Eighteen triterpenoids | synthetic fibrous alumina | a. hexane-benzene (8:1) b. cyclohexane-EtOAc (85:15) c. benzene d. EtOEt e. EtOEt-EtOH (98:2) | a. 2,4-DNPH b. SbCl ₅ in CCl ₄ c. Rhodamine B | diagnostic | 165 |

Thin-Layer Chromatography

TABLE II.21—*Cont.*

| | Compound | Adsorbent | Developer | Visualization | Comments | Ref. |
|----|--|--|--|-------------------------------------|---------------------------------|------------|
| 7 | Sixteen triterpenoids | Silica Gel G | a. isopropyl ether-acetone (5:2)(19:1) b. isopropyl ether c. cyclohexane d. benzene e. CH_2Cl_2 | chlorosulfonic acid-HOAc | diagnostic | 406 407 |
| | Twenty-three triterpene acids | — | f. sol. a g. sol. a plus 5% pyridine h. EtOAc-MeOH-diethylamine (14:4:3) i. chlorobenzene-HOAc (9:1) j. CH_2Cl_2 -pyridine (7:2) | — | — | — |
| 8 | Triterpene acids | Silica Gel G | EtOEt-hexane (1:1) | 50% H_2SO_4 | diagnostic | 421 |
| 9 | Triterpene acids from <i>Cammidiaria glandulosa</i> Schinz, as methyl esters | — | CHCl ₃ -EtOAc (4:1) | conc. H_2SO_4 | diagnostic | 402 |
| 10 | Carotenoids | a. Silica Gel G-Ca(OH) ₂ (1:4) b. Silica Gel G-Ca(OH) ₂ (1:4) impregnated with paraffin | a. benzene-pet. ether (1:1) b. sol. a with 1% MeOH c. MeOH sat. with paraffin | colored compounds | a. diagnostic b. preparative | 446 449 |
| 11 | Isomeric methols | Silica Gel G | a. benzene b. MeOH c. benzene-MeOH (95:5)(75:25) | SbCl_5 in CCl ₄ | diagnostic | 299 |
| 12 | Ipomeamarone | starch-bound silica gel | hexane-EtOAc (9:1) | 2,4-DNPH | quantitative | 2,76 |

| | | | | | | |
|----|-------------------------------|---|--------------------------|-----------------|------------|-----|
| 13 | Isophytol and geranyl-linalol | starch-bound silica gel | hexane-EtOAc (85:15) | | diagnostic | 81 |
| 14 | Azulene and cadalene | a. starch-bound silica gel b. starch-bound alumina | a. pet. ether b. EtOH | trinitrobenzene | diagnostic | 108 |
| 15 | Terpenoids of mint oils | see "Essential Oils," No. 11 | | | | 20 |

Thin-Layer Chromatography

TABLE 11.22. VITAMINS

| | Compound | Adsorbent | Developer | Visualization | Comments | Ref. |
|---|---|---|--|---|--|------------|
| 1 | Carotenes and fat-soluble vitamins A, D, E, K | unbound aluminum oxide | a. MeOH b. CCl ₄ c. xylene d. eleven others | a. 70% HClO ₄ b. conc. H ₂ SO ₄ | diagnostic | 70 |
| 2 | Carotenes and Vitamin A | unbound aluminum oxide | pet. ether | a. SbCl ₃ in CHCl ₃ b. colored compounds | a. diagnostic b. quantitative | 210 211 |
| 3 | B vitamins | Silica Gel G with fluorescent material | HOAc-acetone-MeOH-benzene (5:5:20:70) | a. U.V. b. potassium iodoplatinum dichloroquinone-chlorimide-NH ₃ c. ninhydrin | a. diagnostic b. carried out in dark | 116 |
| 4 | Cyanocobalamin and hydroxycobalamin | Silica Gel G | a. MeOH b. PrOH c. BuOH-HOAc-H ₂ O (20:5:1) (20:5:5) and others d. BuOH-HOAc-MeOH (20:10: 25) e. sixteen others | a. microbiologically b. spectrophotometrically | a. diagnostic b. quantitative | 55 |
| 5 | Vitamins K ₂₍₂₀₎ , K ₂₍₁₀₎ and K ₂₍₁₀₎ | Silica Gel G with sodium fluorescein | a. heptane-benzene (1:1) dried and followed by benzene | U.V. | a. diagnostic b. radioactive c. quantitative | 35 |
| 6 | Tocopherol mixtures | a. Silica Gel G b. Aluminum Oxide D5 | a. CHCl ₃ b. benzene | a. ceric sulfate b. phosphomolybdic acid-NH ₃ | a. diagnostic b. quantitative | 354 |

| | | | | | |
|----|----------------------|---|--|---|------------|
| 7 | Retinen | a. Silica Gel G | a. Pet. ether-benzene mixtures b. MeOH sat. with paraffin | rhodanin | 448 |
| | | b. Silica Gel G impregnated with paraffin | | | |
| 8 | Fat-soluble vitamins | alumina | a. HClO ₄ -70% b. H ₂ SO ₄ -98% | 37 | 431 |
| | | | | | |
| 9 | Ubiquinones | a. Silica Gel G | a. Benzene-CHCl ₃ (1:1) b. acetone-paraffin (sat. with H ₂ O) (9:1) | a. Rhodamine B b. SbCl ₃ in CHCl ₃ | diagnostic |
| | | b. Silica Gel G impregnated with paraffin | | | |
| 10 | Vitamins | | | | 280 |

TABLE 11.23. MISCELLANEOUS

| Compound | Adsorbent | Developer | Visualization | Comments | Ref. |
|--|--|---|--|--|------------------|
| 1 Benzquinones from <i>Myriapodes</i> | starch-bound silicic acid | hexane-EtOAc (83:15) | | a. diazotized sulfanilic acid b. phosphomolybdic acid | diagnostic 12 |
| 2 Antioxidants (phenolic) | unbound polyamide powder | MeOH-acetone mixtures | a. diazotized sulfanilic acid b. phosphomolybdic acid | a. monitored column b. diagnostic | 72 |
| 3 Anioxidants, synthetic | Silica Gel G | a. benzene b. CHCl ₃ c. CHCl ₃ -methyl cellosolve (80:20) | a. phosphomolybdic acid b. diazotized sulfanilic acid c. 2,6-dichloroquinonemchlorimide, neutral and buffered | a. diagnostic b. two dimensional c. quantitative | 352 |
| 4 Alkyl phosphenosides, sulfides, sulfones, and sulfoxides | Silica Gel G | a. acetone b. CHCl ₃ | a. Na ₂ Cr ₂ O ₇ -H ₂ SO ₄ b. KMnO ₄ -H ₂ SO ₄ c. FeCl ₃ and K ₃ Fe(CN) ₆ | diagnostic | 98 |
| 5 Thiophene derivatives | a. Silica Gel G b. Aluminum Oxide G | a. benzene-CHCl ₃ (9:1) b. MeOH c. pet. ether | a. U.V. b. isatin in H ₂ SO ₄ | diagnostic | 65 |
| 6 Aromatic amines and nitrocompounds | see "Amines," No. 5 | | | | 110 |
| 7 Adulteration of the drug <i>Radix Zingiberiae</i> DAB6 | Silica Gel G | CHCl ₃ | SiO ₂ in CCl ₄ | diagnostic | 161 |
| 8 Diphenyl in citrus fruits | starch-bound silicic acid with phosphors | pet. ether | U.V. | quantitative | 194 389 |
| 9 Polycyclic aromatics | acetylated cellulose powder | MeOH-EtOEt-H ₂ O (4:4:1) | fluorescein | diagnostic | 82 |
| 10 Nitramine explosives | Silica Gel G | pet. ether-acetone (2:102) | diphenylamine | diagnostic | 216 |

| | | | | | | |
|----|--|--|---|--|--|-----|
| 11 | Peroxides | starch-bound silica gel | CCl ₄ -acetone (2:1) | a. KI b. ferrous ammonium sulfide and potassium thiocyanate | diagnostic | 242 |
| 12 | Piperidoles, quinolones and cyclohexanedi-ones | unbound aluminum oxide | a. benzene-acetone (1:1) b. acetone-MeOH (3:1) | a. I ₂ b. U.V. | tested procedure | 254 |
| 13 | Honeybee larval foods | silicic acid | MeOH-CHCl ₃ (29:71) | a. p-anisidine b. ninhydrin c. FeCl ₃ d. I ₂ e. conc. H ₂ SO ₄ | diagnostic | 292 |
| 14 | Plasticizers (phthalates, phosphates and other esters) | Silica Gel G with fluorescent material | a. isoctane-EtOAc (90:10) b. benzene-EtOAc (95:5) c. butyl ether-hexane (80:20) | a. phosphomolybdic acid b. resorcinol-EtOH-ZnCl ₂ followed by H ₂ SO ₄ and KOH c. alcoholic KOH followed by urea d. 2,6-dichloroquinonemchlorimide followed by borax e. five others | diagnostic | 293 |
| 15 | Phloroglucinol-butanones from <i>Dryophytes</i> | a. Silica Gel G buffered with NaOAc b. Silica Gel G impregnated with paraffin | a. EtOAc b. MeOH-formic acid-H ₂ O (75:10:15) | Folin-Ciocalteau reagent-NH ₃ | diagnostic | 380 |
| 16 | Chamazulene derivatives and dyes as test substances | a. silicic acid b. starch-bound silicic acid | hexane-EtOAc (9:1) | | methods exploration | 381 |
| 17 | Uronic acid | Silica Gel G | a. benzene b. MeOH | a. U.V. b. FeCl ₃ | a. diagnostic b. on microscope slides | 437 |
| | Aminopyrine | | | | | |

TABLE 11.23—*Cont.*

| Compound | Adsorbent | Developer | Visualization | Comments | Ref. |
|--|--|---|---|----------------------------------|------|
| 18 Neuraminic acid derivatives | Silica Gel G | a. PrOH-1 N NH ₄ OAc-H ₂ O (6:2:1) b. PrOH-H ₂ O (7:3) | a. Bial's reagent b. thiobarbituric acid-HIO ₄ -ethylene glycol | diagnostic | 127 |
| 19 Narcotics in forensic chemistry | Silica Gel G with fluorescent material | piperidine-pet. ether (1:5) | U.V. | diagnostic in urine | 92 |
| 20 Lumiflavin derivatives | Silica Gel G | a. 2 N HClO ₄ -dimethylsulfoxide-BuOH (12:2:6) b. HOAc-H ₂ O-BuOH (2:2:6) | colored compounds | diagnostic | 143 |
| 21 Bufadienolides from toad poisons | Silica Gel G | a. EtOAc b. EtOAc-cyclohexane (80:20) c. EtOAc-acetone (90:10) d. EtOAc sat. with H ₂ O | SbCl ₃ in CHCl ₃ | diagnostic | 457 |
| 22 Adrenaline and its derivatives | Silica Gel G | CHCl ₃ -MeOH (9:1) | H ₄ PO ₄ -phosphomolybdic acid | a. diagnostic b. quantitative | 434 |
| 23 Soladulcidin, a nitrogen-containing aglycone from glycosides of <i>Solanum dulcamara</i> L. | Silica Gel G | EtOAc-cyclohexane (7:3) | vanillin-H ₂ PO ₄ | diagnostic | 338 |
| 24 Anthocyanins | polyacrylonitrile-polyamide (7:1) | 1-pentanol-PrOH-HOAc-H ₂ O (3:2:2:1) plus 1-2% 1-heptanol | colored compounds | a. diagnostic b. descending | 36 |
| 25 Olefins as mercury salts | Silica Gel G | PrOH-triethylamine-H ₂ O (50:25:25) | diphenylcarbazide | diagnostic | 309 |

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